MORPHOLOGICAL EVALUATION OF LYMPHOCYTES ON PERIPHERAL SMEAR EXAMINATION IN ADULT PATIENTS WITH LYMPHOCYTOSIS AND ITS CLINICAL CORRELATION

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In partial fulfillment of the requirement for the degree of

Doctor of Medicine in Pathology (Branch III)

M.D. (PATHOLOGY)

APRIL 2017

DEPARTMENT OF PATHOLOGY

CHENNAI MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

TRICHY – 621 105.

DECLARATION

I solemnly declare that the dissertation entitled "**MORPHOLOGICAL EVALUATION OF LYMPHOCYTES ON PERIPHERAL SMEAR EXAMINATION IN ADULT PATIENTS WITH LYMPHOCYTOSIS AND ITS CLINICAL CORRELATION**" is a bonafide research work done by me in the Department of Pathology at Chennai Medical College Hospital & Research Centre, Trichy during the period from August 2014 to July 31st 2016 under the guidance and supervision of DR.V.SARADA, M.D., Professor & H.O.D, Department of Pathology, CMCH&RC, Trichy.

This dissertation is submitted to the Tamilnadu Dr. M.G.R.Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree (Branch III) in Pathology.

I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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This is to certify that the dissertation entitled "MORPHOLOGICAL EVALUATION OF LYMPHOCYTES ON PERIPHERAL SMEAR EXAMINATION IN ADULT PATIENTS WITH LYMPHOCYTOSIS AND ITS CLINICAL CORRELATION" is a record of bonafide work done by DR. SEKAR LALITHA, postgraduate student in the Department of Pathology, during the course of the study (2014-2017), Chennai Medical College Hospital and Research Centre, Trichy under the supervision and guidance of **Dr. V. S[illegible]A, M[illegible].**

This dissertation is a record of original work done by the candidate, Dr. SEKAR LALITHA; this work was carried out by the candidate herself under my supervision.

Dr. V.SARADA M.D.,
Guide & Professor,
Department of Pathology,
CMCH & RC,
Trichy- 621 105.

Dr. V[illegible]A [illegible].,
Pro[illegible]
Department of Pathology,
CMCH & RC,
Trichy- 621 105.

Dr. SUKUMARAN AN[illegible]AI M.[illegible]., [illegible].H.H.M.,
[illegible]
Chennai Medical College Hospital
& Research Centre,
Trichy- 621 105.

CHENNAI MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE

IRUNGALUR, TRICHY – 621 105.
E.Mail : iec.cmchrc@gmail.com, Phone: 0431-3058661,3058817

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[Dr.P.Rajendran]

Member Secretary
INSTITUTIONAL ETHICS COMMITTEE
Chennai Medical College Hospital & Research Centre
Irungalur, Trichy-621 105
Date.....

TO
Dr.Sekar lalitha - I year Post Graduate , Department of Pathology, CMCH&RC, Trichy.
Copy to office

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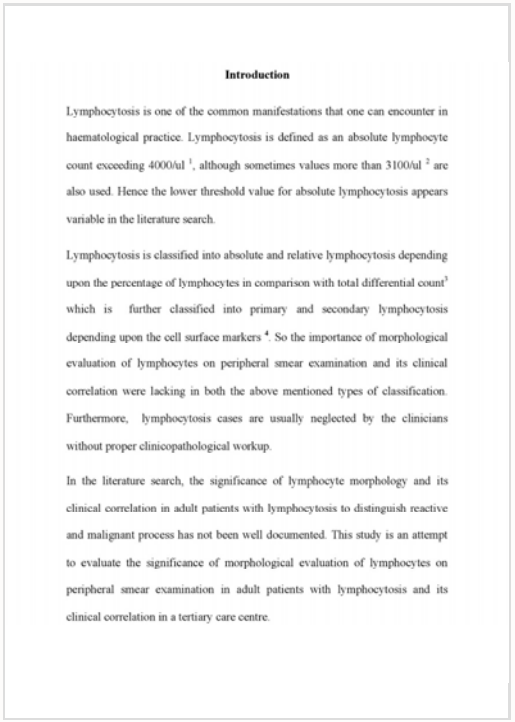
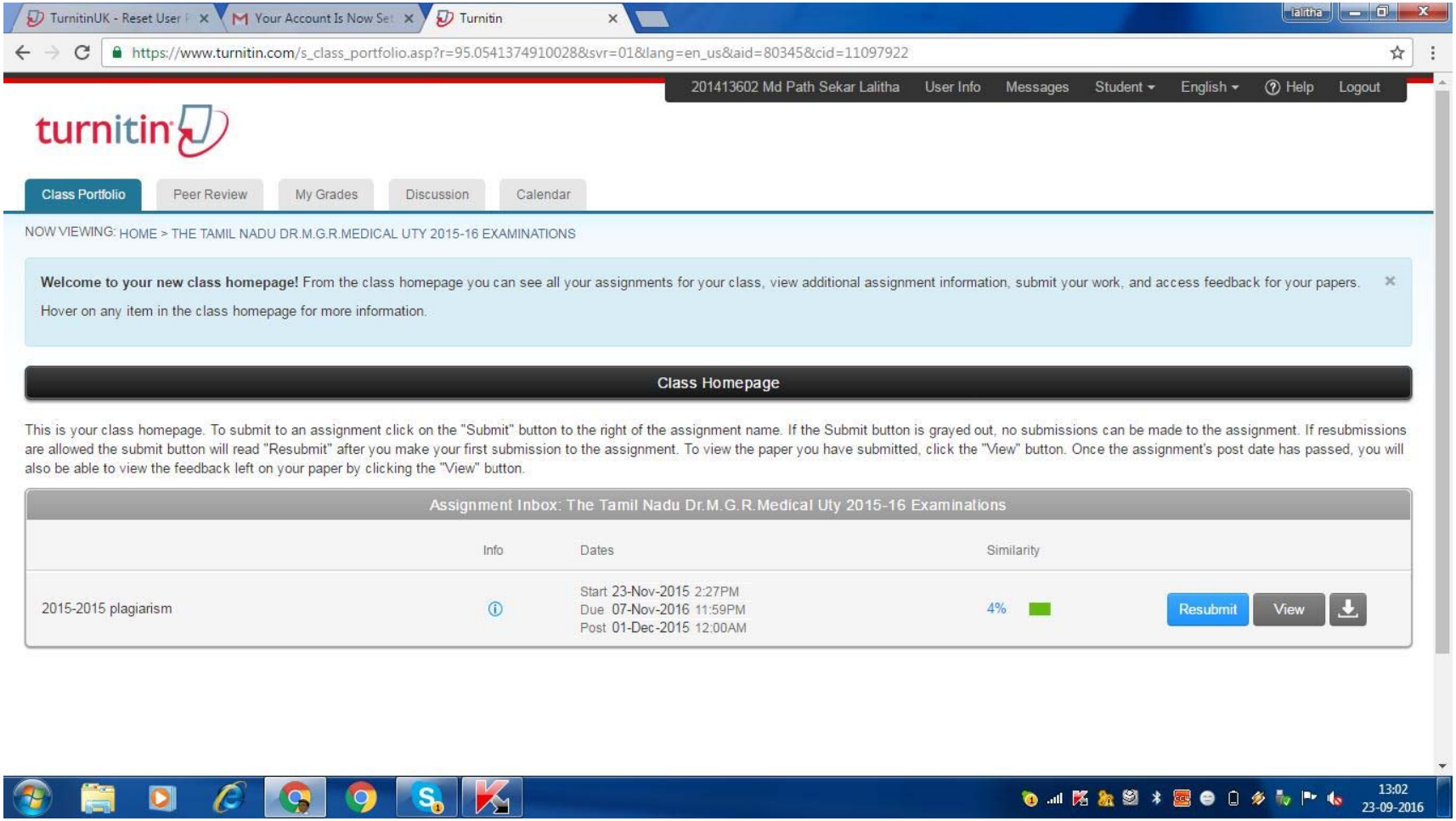


Introduction

Lymphocytosis is one of the common manifestations that one can encounter in haematological practice. Lymphocytosis is defined as an absolute lymphocyte count exceeding 4000/ul [1], although sometimes values more than 3100/ul [2] are also used. Hence the lower threshold value for absolute lymphocytosis appears variable in the literature search.

Lymphocytosis is classified into absolute and relative lymphocytosis depending upon the percentage of lymphocytes in comparison with total differential count[3] which is further classified into primary and secondary lymphocytosis depending upon the cell surface markers [4]. So the importance of morphological evaluation of lymphocytes on peripheral smear examination and its clinical correlation were lacking in both the above mentioned types of classification. Furthermore, lymphocytosis cases are usually neglected by the clinicians without proper clinicopathological workup.

In the literature search, the significance of lymphocyte morphology and its clinical correlation in adult patients with lymphocytosis to distinguish reactive and malignant process has not been well documented. This study is an attempt to evaluate the significance of morphological evaluation of lymphocytes on peripheral smear examination in adult patients with lymphocytosis and its clinical correlation in a tertiary care centre.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LIST OF ABBREVIATIONS

ALC	Absolute lymphocyte count
Plt	Platelet
ul	Microlitre
CD	Cluster differentiation
Ig	Immunoglobulin
CLP	Common lymphoid progenitor pathway
WBC	White blood cells
fl	femtolitre
EBV	Epstien Barr virus
NK	Natural killer cells
CMV	Cytomegalovirus
HIV	Human Immunodeficiency Virus
mm	Millimeter
RBC	Red Blood cell
CBC	Complete blood count
nRBC	Nucleated Red blood cell
EDTA	Ethylene Diamine tetra acetic acid
cumm	Cubic millimetre
H/O	History of
RTA	Road traffic accident
P/V	Per vaginum
HCV	Hepatitis C virus

Introduction

INTRODUCTION

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Aim and Objectives

AIM OF STUDY

To evaluate the morphology of the lymphocytes on peripheral smear examination in adult patients with lymphocytosis and its clinical correlation

OBJECTIVES OF THE STUDY

1) To study the morphology of lymphocytes in adult patients with lymphocytosis

2) To correlate lymphocyte morphology with clinical, haematological and biochemical parameters in the selected cases under study

3) To determine the cut off range of clinically significant lymphocytosis for morphological evaluation

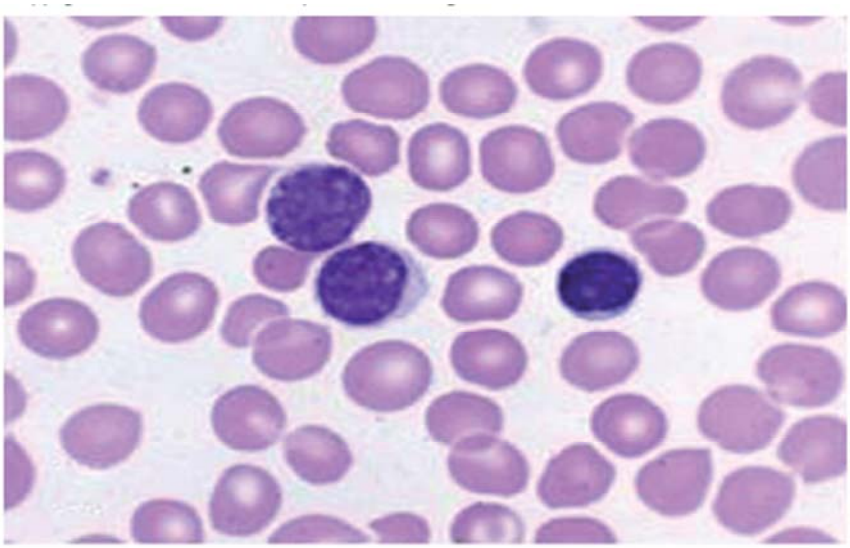
Review of Literature

REVIEW OF LITERATURE

Blood constitutes different types of cells each having a distinctive morphology and function biologically. Lymphocytes are heterogenous type of cells which are different from other cells by their morphology. Lymphocytes are spherical or ovoid with diameter ranging from 6 to 15 um. According to size, the lymphocytes are classified as small lymphocytes and large lymphocytes with sizes ranging from 6 to 9 um and 9 to 15um respectively[4].

Morphologically, lymphocytes are small cells which have an ovoid or kidney shaped nuclei with dense nuclear chromatin occupying majority of the cell area which are stained purple by Romanowsky stain[6]. (FIG 1)

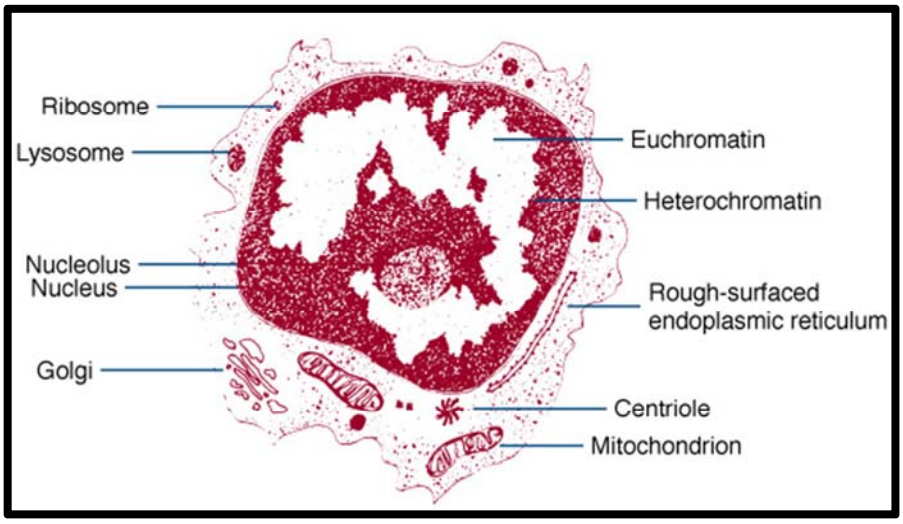
Figure 1 - Morphology of lymphocytes



The lymphocyte in the blood approximately measures 5um in diameter by transmission electron microscopy[4]: nucleus of these lymphocytes have electron dense condensed heterochromatin which shows that that these cells donot proliferate. The nucleoli of these cells are round, measuring from 0.5 to 1.4 um. These nucleoli are composed of three units which are concentrically arranged, the central agranular region, fibrillary region in the middle and a granular zone which contains the intranucleolar chromatin. Nuclear membrane of these lymphocytes contains nuclear pores along with a perinuclear space[38].

The cytoplasm shows the organelles which are typical of eukaryotic cells, with a poorly developed golgi zone along with other organelles like ribosomes , rough endoplasmic reticulum, mitochondria, microtubules, microfilaments and centrioles. Lysosomes in the cytoplasm contain the lysosomal enzymes like acid phosphatase and acid ribonuclease. (FIG 2)

Figure 2 -Diagrammatic representation of lymphocyte



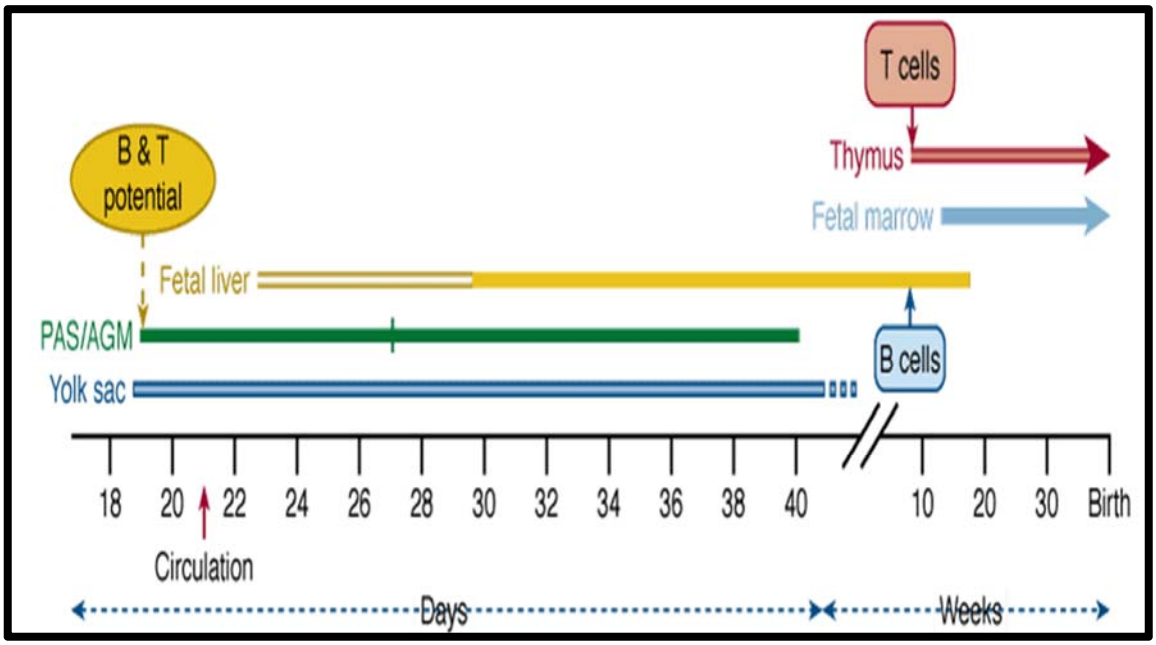
Lymphopoiesis usually occurs later than the myelogenesis and erythrogenesis during embryogeneis. During the 27th day of gestation, there is presence of CD 34+ cells at the aorto - gonad - mesonephros region which initiates lymphoid differentiation into B cells, T cells and Natural killer cells (NK) broadly [4]. Bursa of Fabricius in the marrow is the exclusive site for B cell development from the second trimester. T cells are usually produced in the thymus by prothymocyte migration from the marrow to thymus.

Lymphopoiesis, a constituent of the hematopoietic differentiation is the process with which there is production of the major components of the immnue system which includes the B cells, T cells, NK cells and some of the dendritic cells.

Lymphopoiesis - Prenatal developmental stage

Hematopoiesis begins in yolk sac and is transferred to the embryo proper. Initially it is carried out in paraaortic splanchnopleure (PAS) and aorto-gonad-mesonephros (AGM) regions followed by fetal liver and spleen. Finally the fetal marrow starts the process of hematopoiesis and progresses there on.

Figure 3 - Pictorial representation of human hematopoietic development



Human Hematopoietic Development (FIG-3)

The AGM in humans develops at 27th day of intrauterine life and hematopoiesis takes place till the 40th day. During this stage, human HSCs develops from the endothelium as a cluster two to three cells and subsequently rapidly proliferate. The process continues in the fetal liver till 20 weeks of gestation along with fetal marrow slowly taking its role from the 11th week of gestation itself. CD34+ cells which are found in the marrow of the fetus behaves as true hematopoietic stem cells and generate B cells, T cells and NK cells along with other erythroid and myeloid lineage of cells[4,38] .

Thymus usually starts developing from 4 weeks of gestation and lymphocyte production in the same begins around the 9th week, mature T cells are usually found between 13 to 16th week of gestation .

B cell development

The most important character of a mature B cell is the expression of the heavy or light chain linked immunoglobulin (Ig) on the cell surface exhibiting B cell receptors (BCR). BCR constitutes the immunoglobulin on the cell surface along with its associated signalling molecules which are CD79a and CD79b[4,38].

Progenitor B cells or pro B cells neither have cytoplasmic heavy chains nor the BCR.

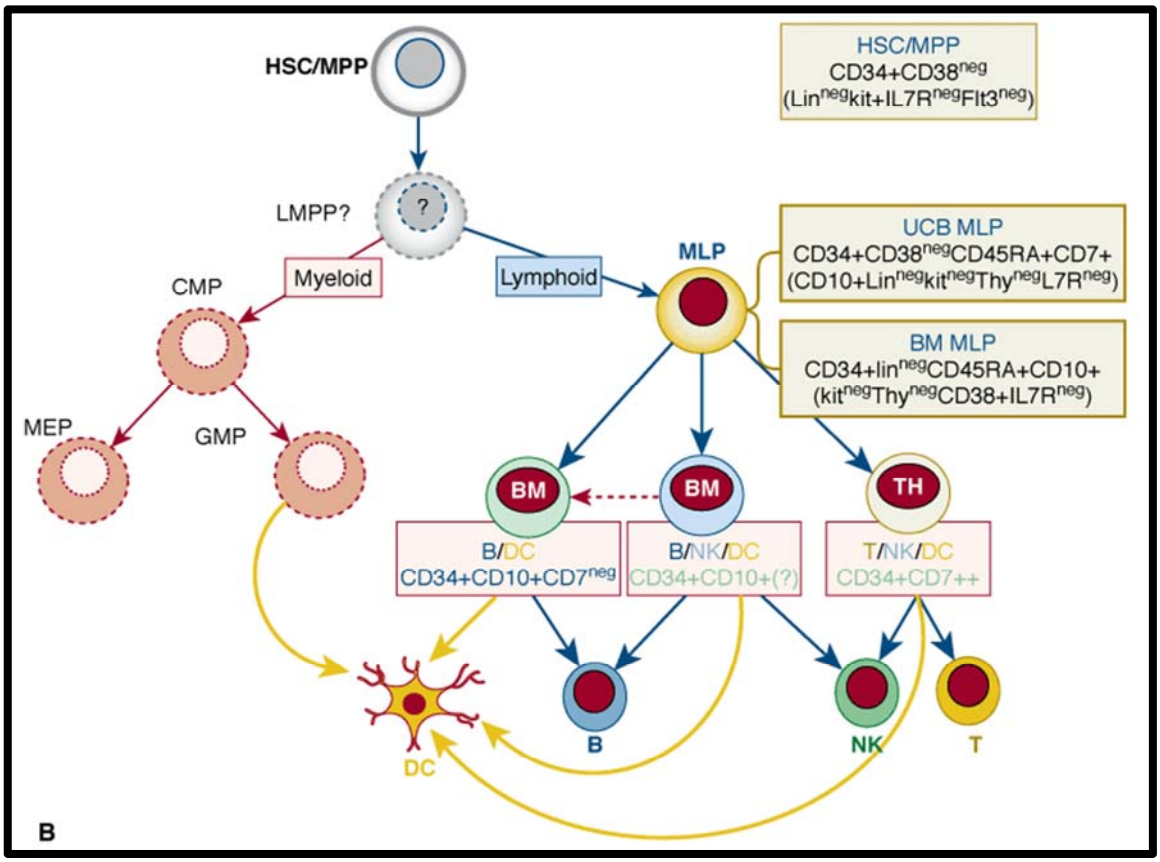
Two stages of B cell development are present; fetal liver and marrow which shows the antigen independant stage and spleen and lymph node with their lymphoid tissue showing the antigen dependant stage.

NK cell or the natural killer cells development occurs between the ninth to the tenth week of gestation . Dendritic cells are produced during all the stages of embryonic and fetal development starting from the yolk sac.

Hematopoietic stem cell enters either of the two pathways; the common lymphoid progenitor pathway(CLP) or from the common myeloid

progenitor (CMP). CLP, a single cell can give rise to all of the lymphoid cells which includes B cells, T cells and NK cells. CLP term is replaced by the terminology MLP - multilymphoid progenitor.

Figure 4 - Human lymphoid progenitor pathway



Human Lymphoid Progenitor (FIG-4) includes CD34+, cell surface marker which is expressed on all human hematopoietic stem cells which along with other markers like CD10 , CD7 and CD45RA[4] helps in identification of the lymphoid type of development.

When there is complete loss or functional loss of any of the WBC, it can lead on to serious health issues, thereby making normal lymphopoeisis

an essential part of healthy life. The mature lymphocytes play a integral part in the immune system which must be continuously produced during life by cell division from the common lymphoid progenitor cells, which when fails leads on to procurement of infections. Lymphopoiesis is one process that needs to be continued throughout life and hence the progenitor cells and their respective parent stem cells must always be present in the body[4].

Activation of lymphocytes happens when either of the types of the lymphocytes, the T or B cells are triggered by antigen - specific receptors on the surface of their cells. This leads on to cell proliferation as well as differentiation into specific effector cells like activated B cells which further gives antibody producing cells and some of the activated T cells changes themselves into cytotoxic T cells[44].

Morphologic Changes Associated with Activation

Stimulation of the lymphocytes is by a series of biochemical along with morphological events. Activating the B and T lymphocytes transforms the resting small lymphocyte into large cells (proliferating cells) with abundant basophilic cytoplasm, a condensed or smudgy type of chromatin sometimes showing a slightly irregular nuclear outline. Nucleoli are inconspicuous.

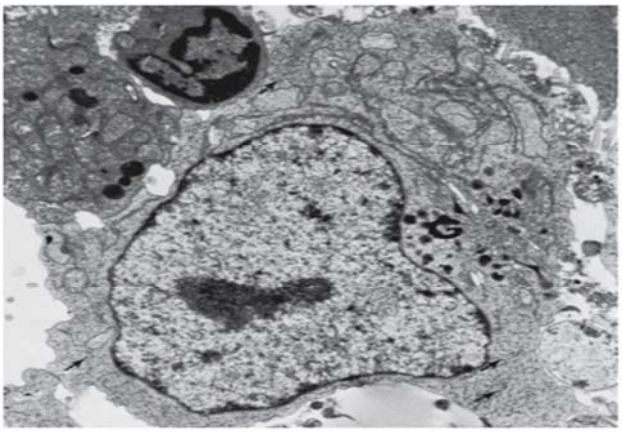
The B. pertussis with absolute lymphocytosis show characteristically activated lymphocytes which are small with nuclei showing cleaving, a mature type of chromatin and scanty cytoplasm[50].

With activation of lymphocytes, the lymphocytes proliferate to memory cells and effector cells, These effector cells are cytolytic T cells, helper T cells and plasma cells. Agents like bacterial products and enzymes causes mitosis therby activating the lymphocyte and are termed as mitogens. Mitogens can be specific for either of the T or B lymphocyte and can activate both of them[4].

Once mitogen stimulation occurs, 4 hours later , the stimulated lymphocytes shows increase in size of the nucleoli with increased concentration and number of the granules present in the granular zone. After this, there in increase in size of the fibrillar zone and the intranucleolar chromatin increases[4,38]. The cytoplasmic size keeps increasing with increase in the number of the organelles present in the cytoplasm .

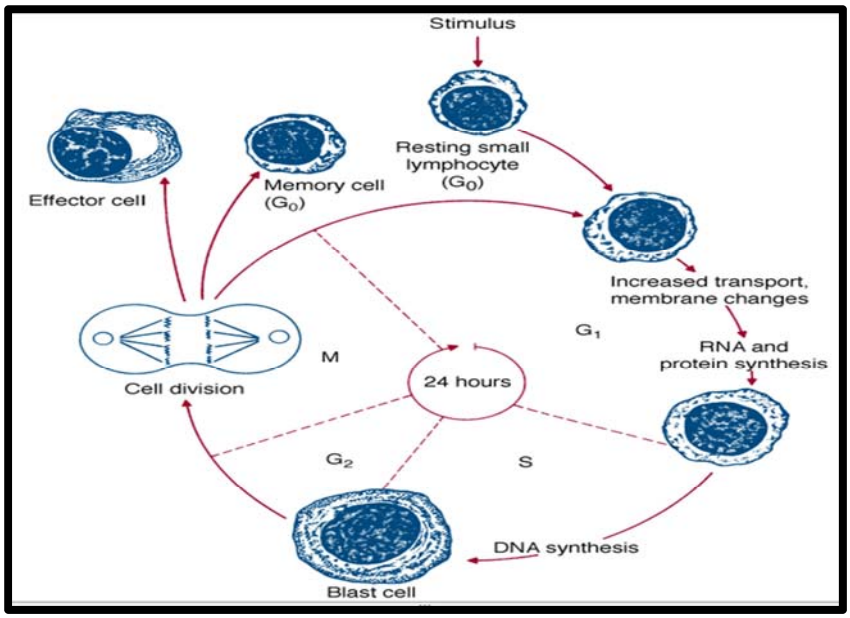
Electron microscopy (FIG 5) shows a euchromatic nucleus, large golgi zone along with many ribosomal aggregrates.

Figure 5 - Electron microscopy photograph of lymphocyte



Once these lymphocytes are activated, they usually enter the cell cycle and follow one of the two pathways; either these lymphocytes undergo many mitotic divisions and return to G0 phase, these lymphocytes are similar in morphology to the non activated lymphocyte[4]. Some become memory cells, which remember the antigen which stimulated the mitosis, or they follow other pathway to become the effector cells which can either be plasma cells or the cytotoxic type of Tcells. (FIG 6)

Figure 6 - Activation of lymphocyte

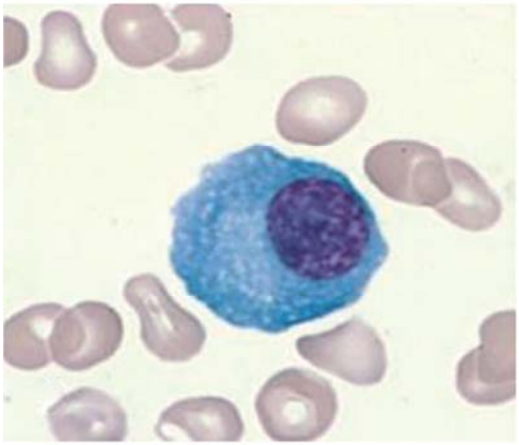


Plasma Cells

Activation of B lymphocyte produces plasma cells. These plasma cells are very characteristic, having abundant cytoplasm along with secretoy immunoglobulins. Resting lymphocyte undergoes many mitotic divisions during the process of cellular differentiation to form a plasmablast[4,38], thereby forming immature plasma cells. With an antigenic response, these immature plasma cells again undergo mitosis at the medullary cords of the lymph nodes which successively gets transformed into a plasma cell which is a mature cell producing antibody.

The plasma cells has a basophilic type of cytoplasm with an eccentrically placed nucleus when stained with a polychrome stain. Occasionally, there may be one or two nuclei also present. The coarse nuclear heterochromatin is distributed in a pattern resembling spokes of a wheel ie, a cartwheel appearance[4,38].(FIG - 7)

Figure 7 - Plasma cell



Antigens of Human Lymphocyte

B lymphocyte antigen

CD20, CD22 and Pax5 are found in these cells. Pax5 is a transcription factor which is the master regulator for the development of of the B cells. This Pax 5 is one such antigen which is expressed from the precursor stage upto the transformation into a plasma cell. Demonstration of monoclonal surface Ig allows diagnosis of clonal, neoplastic B cells. CD19 which is expressed by all B cells and is known as pan B cell marker. Along with the CD antigens , B cells also express three major types of histocompatibility complexes of class II antigens which are DR, DP and DQ[4,38].

Plasma Cells

Plasma cells individually express CD138 and CD38 and not the B cell antigens like CD20, HLA class II antigens or the Pax5.

Mature T Lymphocytes

The mature T cells usually express either CD4 or the CD8 but never both. Both these antigens acts as coreceptors for T cell activation. Most of the CD4 + T cells exhibit helper functions for the initiation of activation of the cytolytic cells or the B cells[46,47].

CD4 and CD8 Lymphocyte Subsets

CD4 is a member of the Ig supergene family and is made up a single chain transmembrane glycoprotein whereas CD8 is 34-kDa dimeric transmembrane glycoprotein. Most of the T cells express the two alpha and beta subunits of CD8. CD8 recognizes the MHC I and CD4 recognizes MHC II. CD + T cells have helper function , other subsets which includes the T - regulatory or T_{reg} cells creates immune tolerance and the follicular T helper cells which has many unique phenotypes. These T_{reg} cells expresses CD25 and Foxp3 which is a transcription factor. The follicular helper (T- TFH) cells expresses Two antigens, CD10 and CD57[46,38].

Natural Killer Cells

The natural killer cells or the NK cells are effector cells which carry a high potential for spontaneous cytotoxicity for target cells. The NK cells express CD16 and CD56. CD8 is found to be expressed in about 50% of these cells.

Thymocyte

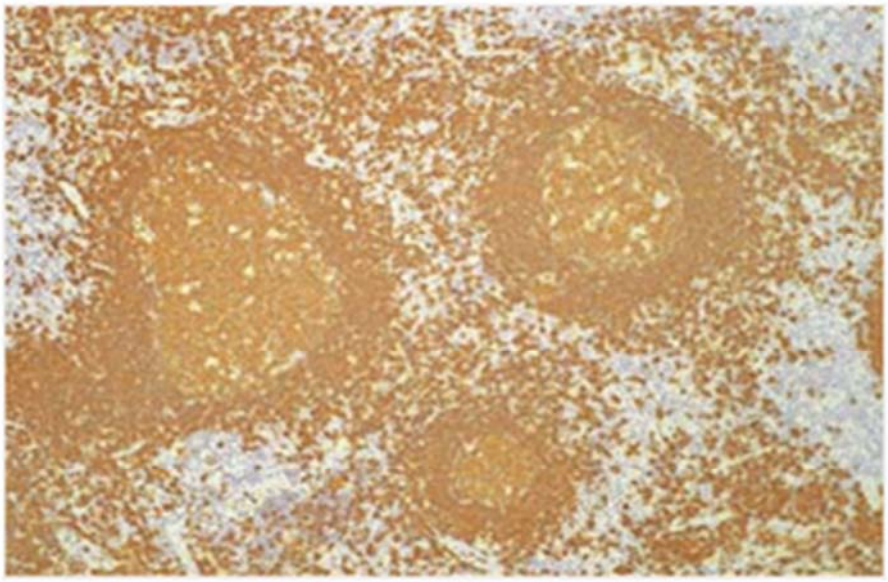
The organ thymus helps in promoting the development of the antigen specific T lymphocytes and also eliminates the T lymphocytes which are self reactive. The thymocytes shows three stages of development, they are single positive, double positive and double negative. The mature single positive cells are located in the medulla whereas the least mature ones which are the double negative cells are found in the

subcapsular areas. These immature T lymphocytes in the subcapsular area express CD2, CD5 and CD7 and these antigens are expressed on all stages of T lymphocyte. In addition, the mature cells in the capsular area express CD1a and cytoplasmic CD3[4,38]. (TABLE 1)

Table 1- Mature Natural Killer cells and T lymphocyte subsets

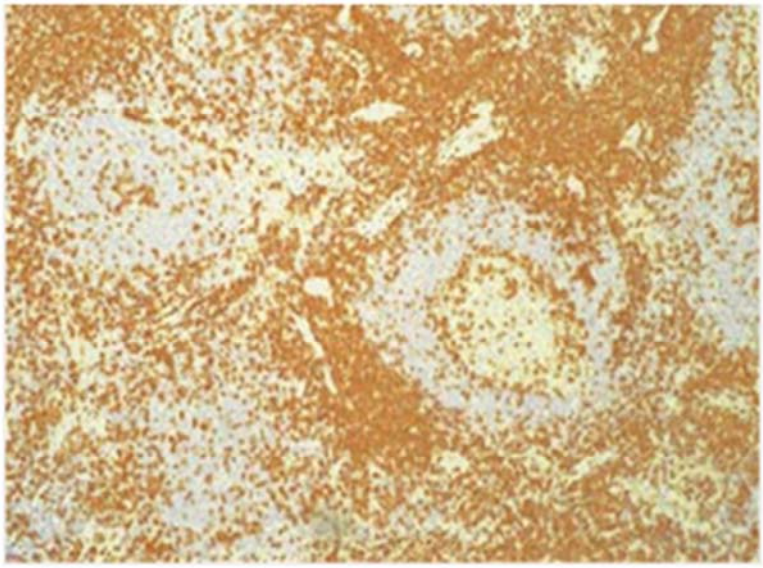
NK or T cell subset	Antigens
CD4+ helper T cells	CD2, CD3, CD4, CD5, CD7, and TCR alpha/ beta
	Subsets express CD10,[49] CD25,[53] CD57,[54] and Foxp3[55]
CD8+ cytolytic T cells	CD2, CD3, CD5, CD7, CD8, and TCR alpha/ beta
	Subsets express CD16,[56] CD56, CD57, cytolytic enzymes[57]
NK cells	CD2, CD7, killer cell immunoglobulin-like receptors (kirs) (multiple)
	Negative for CD3, TCR (alpha/beta or gamma/delta)
	Subsets express either CD16 partial, CD56 bright or CD16 bright, CD56 moderate[58]
	Cytolytic enzymes
Gamma/ delta T cells	CD2, CD3, CD7, TCR gamma/delta
	Usually negative for CD4
	Subsets express CD5, CD8, and cytolytic enzymes

Figure 8 - IHC - CD20 as Pan B cell marker



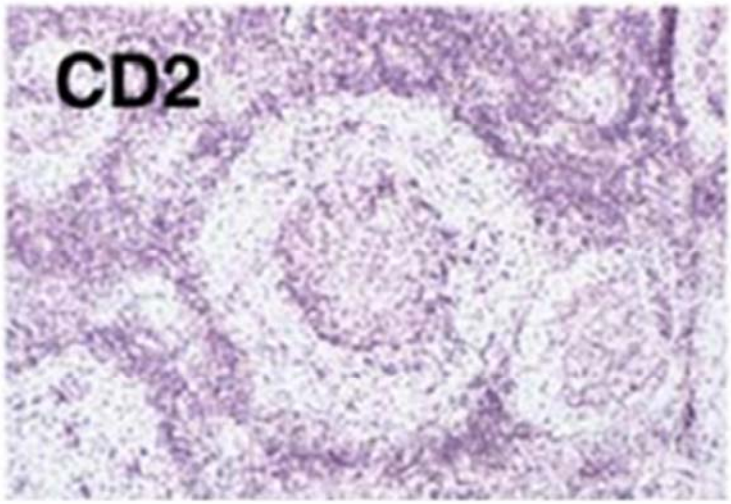
Pan B cell marker - CD20. Stained the follicular germinal centres, mantle zone and scattered B cells in the perifollicular areas.

Figure 9 - IHC - CD3 as Pan T cell marker



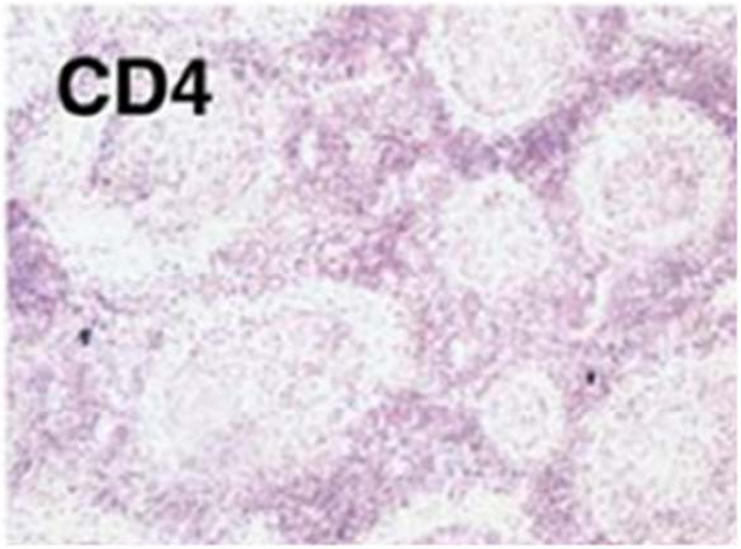
Pan T cell marker - CD3. The perifollicular areas and scattered T cells in the germinal centres have taken up the stain.

Figure 10 - CD2 staining of T cells



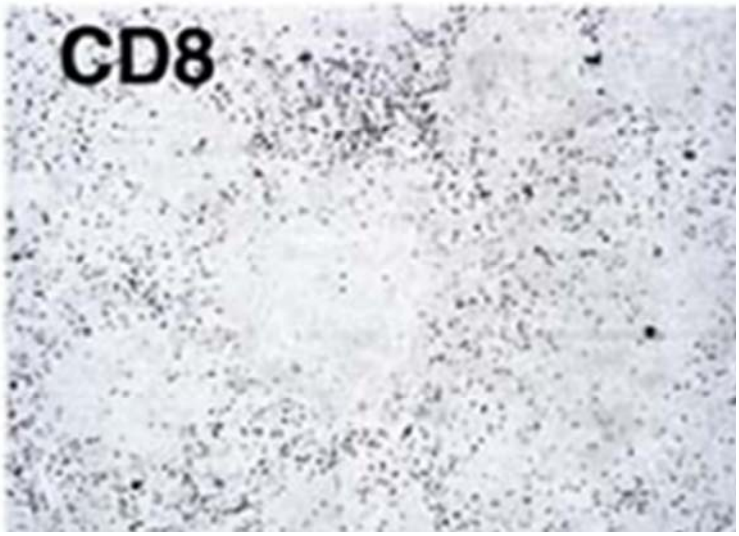
CD2 - all T cells in the interfollicular areas and in the germinal centres have taken up the marker.

Figure 11 - CD4 staining of T helper cells



CD4+ helper T cells are mainly localized in the interfollicular areas and in the germinal centres.

Figure 12 - CD8 staining of cytotoxic T cells



CD8+ cytotoxic T cells, mostly localized to the interfollicular zone.

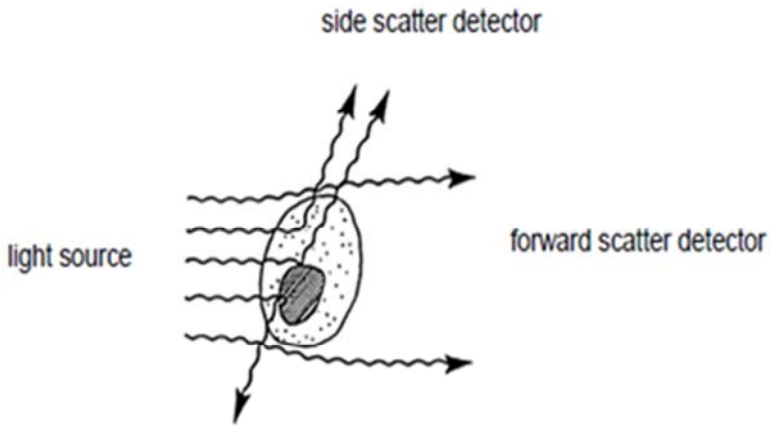
FLOW CYTOMETRY

The flow cytometry is the most effective tool available to define the lymphocyte subset. It is mainly based on the fluorescence principle or due to emission of the light due to release of energy which is attained through absorption of light at various wavelength. Monoclonal dyes are coupled to fluorescent dyes which are subsequently called fluorochrome[4]. This fluorochrome fluoresces with a specific spectrum of light when it is excited with light at a wavelength. These labelled fluorochrome conjugated antibodies are detected by the flow cytometer when they are passed in liquid stream through the laser light beam at specific wavelength. With each cell passing through this laser beam, the laser light scatters and also excites the dye molecule attached to the cell, thereby creating a fluoresce, the scattered light and fluoresce are detected using a sensitive

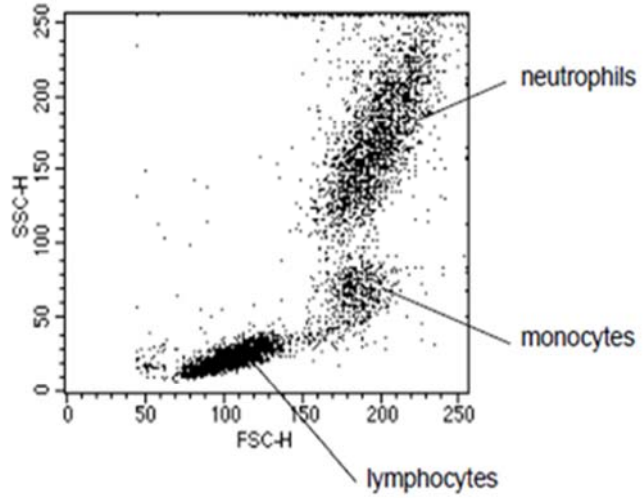
photomultiplier tube[4,38]. Once detected, they give us information on the cell's granularity and to which extent there is binding with the dye. This plays an important way to distinguish the various subsets of the lymphocytes from one another.

The other common use of the flow cytometer is to isolate the lymphocytes which expresses specific surface bound antigens. This is carried out with the help of a fluorescence activated cell sorter with which the fluorescence signals of the various cells are passed through the laser light and further passed on to a computer[4,5,6]. An electric charge is triggered which passes on from nozzle in to the liquid stream at a specific time, breaks up the stream into droplets with the desired cells. These droplets either carry a positive or negative charge which gets deflected from the mainstream when passed between plates containing opposite charge. This helps us distinguish two different subsets of cells from one another and also from the mainstream of unsorted cells which don't show deflection.

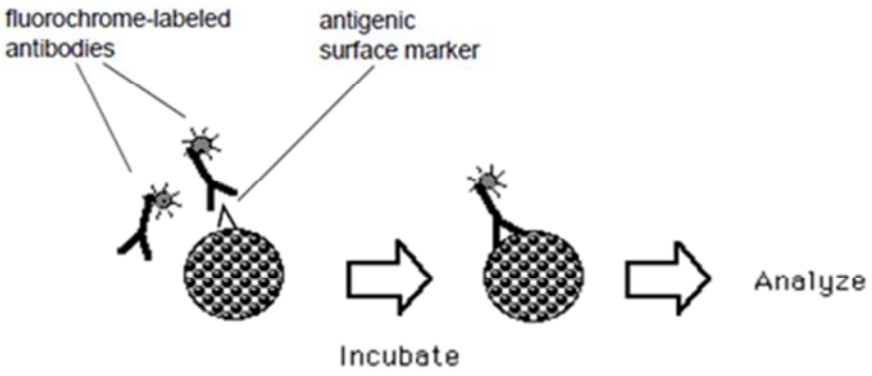
The flow cytometer also helps us to monitor the lymphocyte cell division and to identify the lymphocyte subsets that produces some specific cytokines[4]. By using specific phosphoproteins, this flow cytometer helps us monitor biochemical events that occurs inside the lymphocyte which are mostly triggered by antigen or when there is cross linking of one or more surface receptor molecules.



Light scattering properties of a cell



Cell populations based on forward and side scatter



Specific binding of fluorochrome - labelled antibodies to cell surface antigens

Composition of lymphocyte

Ion and water

A resting lymphocyte has a mean volume of about 200 um ^{3} and has about 71 % of water by weight. Total cation content is about 35 fl/ cell, constituting 22 - 28 fl/cell of potassium and 7.9 fl/cell of sodium approximately[4,38,5]. The volume of the cell is regulated by voltage gate and also by calcium activated potassium channels which is present in the lymphocyte membrane. Any pharmacologic inhibition will block these channels thereby there won't be activation of these lymphocytes. Calcium in these resting lymphocytes is about 580 - 800 pmol/10^6 cells. The free calcium content is usually low in the resting lymphocytes whereas increases to a higher value when there is activation of these lymphocytes[4].

Lymphocyte membrane

The plasma membrane of a lymphocyte is made up of equal parts of protein and also glycospingolipids along with about 6% of carbohydrate. The most predominant phospholipid in the lymphocyte plasma membrane is the phophatidylcholine. The membrane also constitutes saturated fatty acids and the available membrane proteins are glycosylated.

Extracellular membrane associated enzymes - Ectoenzymes

The enzymes exposed on the external surface of the lymphocytes are called ectoenzymes. The various molecules are CD10, CD13, CD26, CD38, CD39, CD73, CD143, CD 156a, CD156b, CD157 and CD224[4,5,38].

Intracellular membrane associated enzymes

B lymphocytes express selectively BTK- bruton tyrosine kinase, one of the tyrosine kinases which has an important action in the transduction of signals. When certain mutations tend to interact and disrupt these enzymes, there is impaired B cell development which further leads on to dysregulation of B cell function or immune deficiency. Whereas, T cell development mainly depends upon cytoplasmic receptor associated tyrosine kinase like 70kDa (ZAP-70) and lck - leukocyte tyrosine kinase. These enzymes tend to interact with the cytoplasmic domains of different accessory molecules which includes CD2, CD4, CD8, CD50 and CD 4^4. Due to these interactions, the receptor tyrosine kinases plays a major role in signal transduction in immune interactions.

Cytomarix

Lying below the plasma membrane is the fully developed cytomatrix with various mechanical and structural proteins like tubulin, actin, myosin, tropomyosin, filamin and spectrin like molecules. These are important during intercellular interactions for formation of synapses. When a lymphocyte is activated either by antigens or sometimes mitogens, it leads onto changes in the interaction between membrane components and the cytoskeleton which further leads to processing of antigen, secretion of immunoglobulin or even cytotoxic reactions which are cell mediated.

Organelles

A resting lymphocyte usually has few ribosomes and scanty endoplasmic reticulum, whereas in plasma cells which are an end product of B cell differentiation shows a well developed rough endoplasmic reticulum and golgi apparatus and abundant RNA suggesting the increased levels of immunoglobulin synthesis and low DNA content.

Cytoplasmic granules

Cytotoxic T lymphocytes and the Natural killer cells are the ones which contain abundant cytoplasmic granules than the other lymphocytes. The main enzymes in these are perforin which is a pore forming proteolytic enzyme, and the other is granzyme which is a preinase with proapoptotic activity[5]. The perforin /granzyme is the main system upon which a cytotoxic lymphocyte depends on to kill the target. Both of these enzymes are not in an active state in a normal cytotoxic T lymphocyte.

Lymphocytosis carries a definition of absolute lymphocyte count exceeding 4×10^9 / L (1). Lymphocytosis can be divided into primary and secondary , primary being leukemic and secondary constitutes the reactive forms. Primary lymphocytosis occurs due to the intrinsic defect seen in the expanded lymphocyte population which cause absolute lymphocytosis[4,38]. Together, these conditions are known as lymphoproliferative disorders occurring secondary to accumulation of

monoclonal B cells, T cells and NK cells. Causes of primary lymphocytosis are listed below

A) Lymphocytic malignancies

1) Acute lymphocytic leukemia
2) Chronic lymphocytic leukemia and related disorders
3) Prolymphocytic leukemia
4) Hairy cell leukemia
5) Adult T cell leukemia
6) Leukemic phase of B cell lymphomas
7) Large granular lymphocytic leukemia[4]
    a) Natural killer cell leukemias
    b) CD8+ T- cell large granular lymphocytic leukemia
    c) CD4 + T cell large granulocytic leukemia
    d) Gamma/ Delta T cell large granular lymphocytic leukemia

B) Monoclonal B - cell lymphocytosis

C) Persistent polyclonal B cell lymphocytosis[4]

Monoclonal B cell lymphocytosis shows expansion of monoclonal B cells without any clinical symptoms or signs and these individuals mainly the elderly males are prone to develop Chronic lymphoid

leukemia (CLL) if they carry CLL immunophenotype monoclonal B cell lymphocytosis[4,5,6].

Persistent Polyclonal lymphocytosis of B lymphocytes (PPBL) shows moderate absolute lymphocytosis without an evidence of infection or conditions that can show lymphocytosis. B cells of these PPBL shows higher levels of IgD and CD 27[7]. Affected individuals show mild splenomegaly along with increased serum IgM levels and they are mostly smokers.

Secondary lymphocytosis shows increase in absolute lymphocyte count which is secondary to physiologicaal or pathological responses to infections, cytokines, toxins etc. The casuses of secondary or reactive lymphocytosis are listed below ;

II) Reactive lymphocytosis[4,5,38]

A) Mononucleosis syndromes

1) Epstein - Barr virus
2) Cytomegalovirus
3) Human immunodeficiency virus
4) Herpes simplex virus type II
5) Rubella virus
6) Toxoplasma gondii
7) Adenovirus

8) Infectious hepatitis virus

9) Dengue fever

10) Human herpes virus type 6 and type 8

11) Varicella zoster virus

B) Bordetella virus

C) NK cell lymphocytosis

D) Stress lymphocytosis (acute)

1) Cardiovascular collapse

a) Acute cardiac failure

b) Myocardial infarction

2) Staphylococcal toxic shock syndrome

3) Drug induced

4) Major surgeries

5) Sickle cell crisis

6) Status epilepticus

7) Trauma

E) Hypersensitivity reactions

1) Insect bite

2) Drugs

F) Persistent lymphocytosis (subacute or chronic)

1) Cancer

2) Cigarette smoking

3) Hyposplenism

4) Chronic infection

a) Leishmaniasis

b) Leprosy

c) Strongyloidiasis

5) Thymoma

Infectious mononucleosis , usually occurring secondary to Epstein- Barr virus (EBV) , show atypical lymphocytes which show polyclonal population of CD8 + T cells, CD16+, CD56+, NK without any change in CD4+ T cell and CD19+ B cells[6,7].

Bordetella Pertussis, shows increased absolute lymphocyte count ranging between 8 to 70 x 10^9/ L due to inability of lymphocytes to travel from the blood to lymphoid tissue due to presence of pertussis toxin which inhibits chemokine receptors. Morphologically these lymphocytes predominantly show clefting of the nucleus.

Large granular lymphocytosis results primarily due to expansion of CD8 + T cells and NK cells representing an increased response to systemic infections, associations with rheumatoid arthritis and severe

aplastic anaemia[4,5,6] are also seen in literature. Some cases of Philadelphia chromosome positive leukemia on treatment with dasatinib have shown NK lymphocytosis[4] T cell large granular lymphocytosis can also be an indicator of T cell large granular lymphocytic leukemia.

Stress lymphocytosis with the above mentioned causes can show an absolute lymphocytosis more than 5 x 10^9 /L and reduces to normal levels within hours[26]. Transient lymphocytosis occurs due to adrenaline release or given during medical practice and shows a characteristic two phase reaction , quick lymphocyte mobilization occurring within 30 minutes , following which granulocyte count increases with decreasing levels of lymphocytes. The transient absolute lymphocytosis mainly occurs in trauma, cardiac conditions, sickle crisis, abdominal pain and obstetric emergencies . This type of transient lymphocytosis usually resolves within 24 to 48 hours of diagnosis. There are also studies by Nitin J. Karandikaret et al which states that stress induced lymphocytosis, if not resolving in a maximum of 48 hours, can help us reveal if any associated clonal disorder is present by flow cytometric evaluation. Also discussed in the article is the morphology of these lymphocytes during an absolute lymphocytosis showing a different morphological appearance than the different morphologies seen with viral infections.

Hypersensitivity reactions usually associated with large granular lymphocytosis and lymphadenopathy are mostly due a delayed hypersensitivity reaction caused by insect bite[4,5]. Some drug reactions can also cause subacute lymphocytosis mostly 2 - 8 weeks after initiating the drug.

In cancer, usually patients with lymphocytosis might have an underlying neoplastic disorder. In malignant thymoma, there is usually a persistent T cell lymphocytosis which is mostly due to the thymic hormone release.

Lymphocytosis is usually seen with post splenectomy patients and it persists for very prolonged periods and shows an absolute lymphocyte count between 4 to 8 x 10^9 /L.

Mononucleosis syndromes

The term was introduced for acute, self limiting syndrome of mononuclear leukocytosis in febrile patients. The most common cause of infectious mononucleosisis Epstein Barr virus and the members of the herpes family which also causes febrile syndrome with peripheral blood lymphocytosis[42]. Other causes of lymphocytosis with febrile illness are human immunodeficiency virus, human herpes virus - 6, rubella, hepatitis A, adenovirus, toxoplasma gondii.

Epstein Barr virus mononucleosis

Transmission is mainly by close contact and increased incidence during the summer. Lower socioeconomic status people are more commonly infected. Reactivation occurs frequently which is a main cause for prevalence of disease in all age groups.

Virology and pathogenesis

EBV or Epstein barr virus belongs to the gammaherpesvirinae subfamily and is a DNA virus. Initially, the virus infects the memory B cells followed by shedding of virus in the oral secretions and genetic factors associated with the infection are interleukin 10- 592/A and gamma plus 874 T/A. The latter has a allele T/T which shows a severe course than the T/A allele and the other interleukin[4,42].

With the syndrome, within few days post infection, an exuberant cytotoxic T cell response follows as the T lymphocytes recognize the viral antigens on the infected B cells as foreign antigens.

Clinical manifestations:

In young, there is predominantly respiratory tract infection. Other manifestations include otitis media, pharyngitis, gastroenteritis, rashes, eyelid or periorbital swelling. In an age group of 12 - 25 years, by 30 - 45 days of infections, the person develops fever and tiredness initially, followed by pharyngitis and tonsillitis which can be of severe grade.

Infection mainly occurs due to attachment of the virus to the cell surface CD21 glycoprotein. The EBV infection causes proliferation of the infected B cells in the pharyngeal nodes, from here, the virus travels into the lymph circulation, thereby causing a huge T lymphocyte response which reflects as peripheral blood lymphocytosis with reactive forms along with lymphadenopathy, splenomegaly and also hepatic inflammation due to trapping of CD4 + and CD8 + lymphocytes in liver causing cytokine release and results in inflammation.

Lab findings

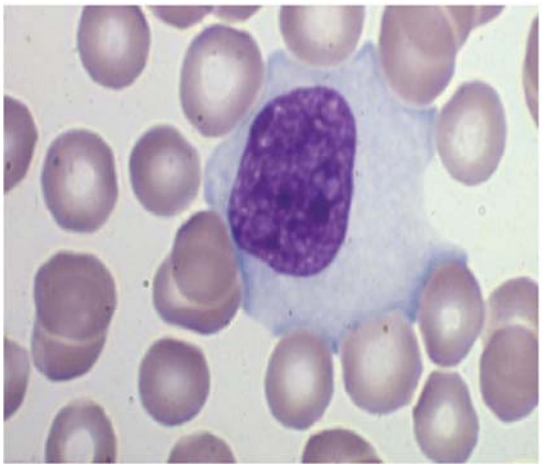
Antibody responses

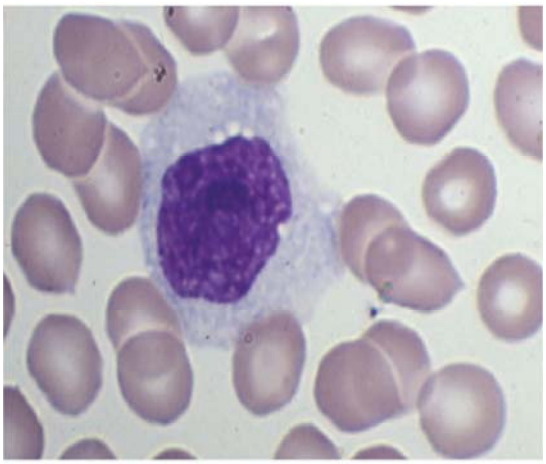
IgM and IgG antibodies to EBV capsid antigen are detectable (VCA), followed by development of antibody to early antigen (EA).

Reactive lymphocytosis

Lymphocytosis occurs due to expansion of the cytotoxic T lymphocytes. The reactive lymphocytes found on the peripheral blood smear examination are larger than the traditional lymphocytes, may have a vacuolated cytoplasm, could be lobulated and with a eccentrically placed nuclei. Sometimes, the cell membrane can appear indented due to the neighbouring erythrocytes. Cytoplasm shows peripheral dark staining which is termed as skirting.(FIG 13,14)

Figure 13- Reactive lymphocyte **Figure 14- Reactive lymphocyte**





Complications of EBV mononucleosis

a) Hematologic

Decreased platelet count. Sometimes EBV mononucleosis can lead onto severe form of immune thrombocytopenia, immune hemolytic anemia and also aplastic anemia.

b) Neurologic

Acute form of encephalitis, acute cerebellar ataxia, viral meningitis, acute disseminated encephalomyelitis - Alice in wonderland syndrome and also cranial nerve palsies.

Other complications include chronic fatigability, multiple sclerosis, Systemic lupus erythematosus and rheumatoid arthritis, severe form of the disease can lead onto NK or T cell lymphoproliferative disease[4,5,6,38].

Cytomegalovirus mononucleosis

The most common causes for an young child to get infected by the virus is transmission of mother to baby through breast milk and Mothers Carrying CMV .infection in their cervix also transmits the infection to the newborn infant. Adults acquiring the infection by body fluids, most important being the semen.

Clinical manifestations

High grade fever of with a temperature of 104^0F and palpable spleen. Other constitutional symptoms of weight loss, malaise are also noted.

Lab findings

Neutrophilia is noted with many band forms of neutrophils. Lymphocytosis develops later and is very similar to that of an individual affected by EBV infection. In the CMV infection, the cell affected is the macrophage and lymphocyte is seen as a response to the infection of the macrophages. Differentiating from EBV infection, neither of the exudative pharyngitis, heterophile antibody or polyclonal antibodies occurs in this infection as in EBV. IgM and IgG antibodies to CMV infection are positive during initial stages of the disease. PCR - polymerase chain reaction for CMV is positive and the virus can also be isolated from both the saliva and urine.

Complications

.Hemolytic anemias and thrombocytopenia occurs in primary infection by the cytomegalovirus. Neurologic complication commonly associated is Guillian Barre syndrome. Lymphomas do not occur with this infection due to the mechanism which provokes T cell response which only leads on to reactive lymphocytosis.

Primary CMV infection is a common problem in organ transplantation. CMV primarily infects all organs and can remain in a latent state. Symptomatic infection in a patient can occur when he receives the organ from a seropositive patient and due to the immunocompromised state, there is reactivation of the CMV which was latent in the tissue macrophages. Reactivation makes the tissue behave as a foreign antigen and induces rejection. Also in HIV patients, there is progression of the disease.

Atypical lymphocytes are actually non malignant type of lymphocyte which can be visualised by blood smear examination , it is a reactive form of lymphocyte which is produced with various disorders[38].

Due to the polyclonal type of immune response to the antigenic stimulation, morphology of the lymphocyte varies for various diseases[53]. These atypical lymphocytes appears to be like a cross between a plasma cell and lymphocyte and are called plasmacytoid lymphocyte,

lymphocytoid plasma cell or plasma cell at times. These atypical lymphocytes are usually larger than a typical lymphocyte, with a indentation present at the periphery by the surrounding cell giving a scalloping appearance[4,53,54]. The nucleus of these cells have a slightly finer chromatin and are lobulated or indented , oval, kidney shaped, round, divided appearance and sometimes placed peripherally. These reactive lymphocytes have shown to play an important role in immune response. The atypical lymphocytes seen in infectious mononucleosis have both T and B cell types giving us an information that the the heterogeneity is due to the reactive nature which actively proliferate[53].

The most common causes of atypical lymphocytes are either infections like Epstein barr virus, Cytomegalovirus, toxoplasma, rubella, herpes simplex, hepatitis A, hepatitis B, rubella etc. Other causes include drug and toxic reactions caused by hydantoin drugs, lead, phenothiazine etc. The typical lymphocytes are also seen in post perfusion syndromes, immunizations, radiations, graft rejection commonly seen in renal transplant, malignant hodgkin's disease, certain idiopathic conditions like sarcoidosis, myasthenia gravis, hormonal induced causes including stress, addison's disease and thyrotoxicosis[54]. Autoimmune disorders like SLE, rheumatoid arthritis, autoimmune hemolytic anemia and agammaglobulinemia also causes atypical lymphocytes.

MORPHOLOGY OF MALIGNANT LYMPHOCYTES

Typical Chronic lymphocytic leukemia shows malignant lymphocytes with small nuclei[60], coarse blocky chromatin along with smudge cells.

The malignant morphology of lymphocytes in splenic marginal zone lymphoma, shows villous lymphocyts, which has bipolar cytoplasmic projections[60]. The hairy cells in hairy cell leukemia shows spiky cytoplasmic projections which extends from the periphery of the malignant cell[60].

Typical mantle cell lymphoma shows lymphocytes which are large lymphocytes with folding of nuclei, scanty basophilic cytoplasm along with a single prominent nucleoli sometimes[60]. The blastoid variant of the mantle cell lymphoma shows comparatively large lymphocytes with moderate amount of blue cytoplasm and the nucleus is usually convoluted or indented.

The malignant lymphocytes in a burkitt lymphoma are usually moderately sized cells with a round to oval shaped nuclei and prominent 1-3 nucleoli[60,4]. The cytoplasm of these cells show multiple small vacuoles.

The most abnormal morphology of lymphocytes are seen in large cell lymphomas resembling immunoblasts with prominent 1-2 nucleoli. These cells are large with a deeply basphilic cytoplasm folded nuclear with

occasional vaculations, angulated to folded nuclear membrane[60]. Sometimes, these cells are misinterpreted as blasts, but these large cell lymphoma cells lack the smooth and even chromatin found in blast cells.

Follicular lymphoma involving the blood usually shows a characteristic morphology of lymphocyte. The malignant lymphocytes are slightly bigger than the normal lymphocytes with presence of clefting, coarse chromatin and scanty cytoplasm[60].(FIG 17)

Figure 15: Chronic lymphocytic leukemic cells - small , round cells with blocky chromatin

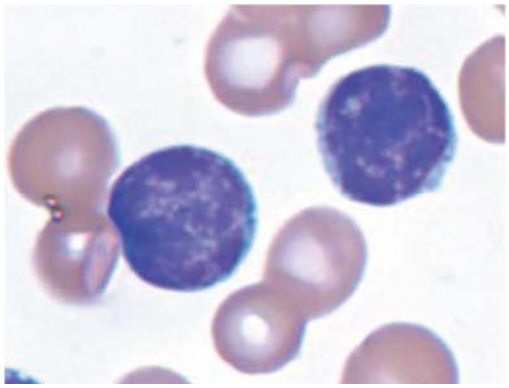


Figure 16 : Splenic marginal zone lymphoma with villous lymphocytes

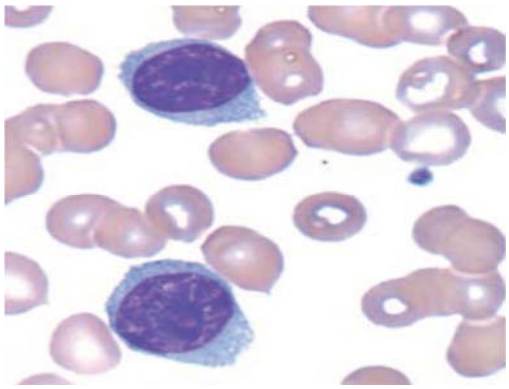
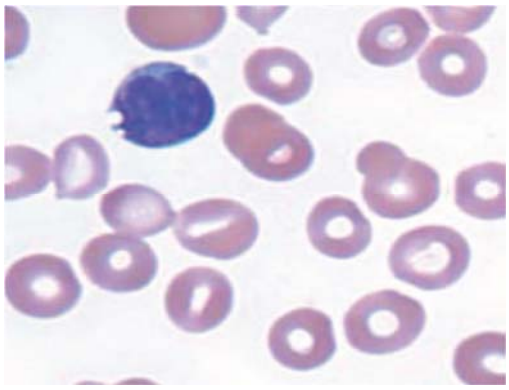
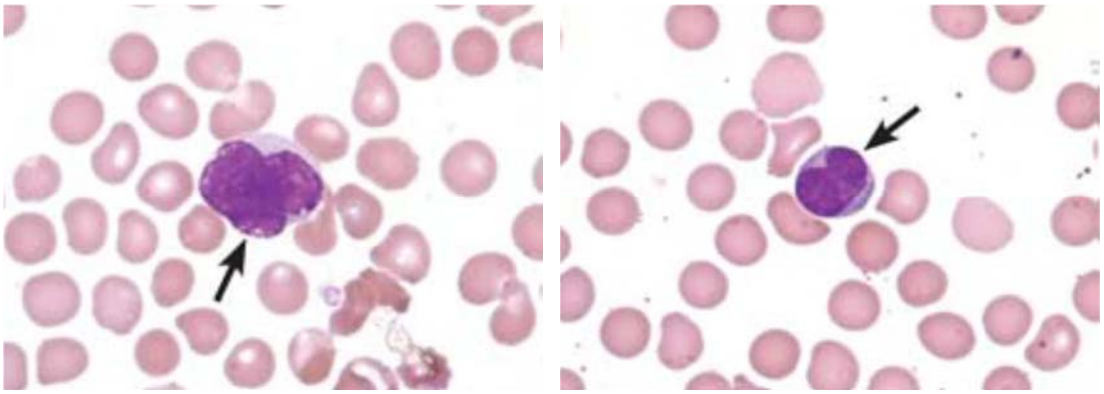


Figure 17: Follicular lymphoma with clefted nucleus



Sezary cells which are associated commonly with malignant lymphoma should be differentiated from a reactive lymphocyte. Nucleus of the sezary cells usually are convoluted, cerebriform[4] or folded with a hyperchromatic nucleus, clumped chromatin, scanty pale blue or blue gray cytoplasm compared to a pale to a deeply basophilic cytoplsam in a reactive lymphocyte with moderate to abundant cytoplasm.

FIGURE 18: SEZARY CELLS - malignant lymphocytes



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PERIPHERAL BLOOD SMEAR EXAMINATION

Examining a blood smear includes evaluating red cells, white cells and morphology of the platelets. A minimum of 8 to 10 fields are reviewed for reporting a peripheral smear under oil immersion. Red cell evaluation includes mainly the morphology reporting the various change in shape, size, colour, its distribution , inclusions and the concentration of hemoglobin[43]. Evaluating the white blood cells should include the the differentiation of the white cells, any nuclear or cytoplasmic abnormalities and inclusions on the WBC should be noted. Platelet count is counter checked with the automated values, along with the shape and size of the platelets and mainly platelet clumping is looked for. Most importantly , a peripheral smear abnormality should be identified whether it is of artefactual or pathological.

Initially the smear is examined under low power, 10x scan; where the quality of staining of the smear is determined followed by distribution of the cells mainly in the edges and centre of the slide for clumps and for abnormal cells at periphery of the smear[6,43]. Area for further examination of the smear is selected such that the RBC's do not touch each other with a graduated central pallor and the selected area must not show many broken cells or precipitation of stain.

High power examination, in 40x includes;

1) Determining the estimated WBC count by counting the number of WBC's in 10 fields and an average of the same is taken. The estimated count is reported according to the values in the following table.(Table 2)

TABLE 2 - Estimated WBC count per high power field (40x)

Number/ high power field	**Estimated Total WBC count in mm^3**
2 - 4	4000 - 7000
4 - 6	7000 - 10,000
6 - 10	10,000 - 13,000
10 - 20	13,000 - 18,000

Total WBC count can also be estimated by multiplying the average number of WBC's determined and 2000. This is based on a fact that each WBC seen in the power 400x is approximately equivalent to 2000 cells/ ul of blood[43].

2) The automated value and WBC estimated through the smear is correlated

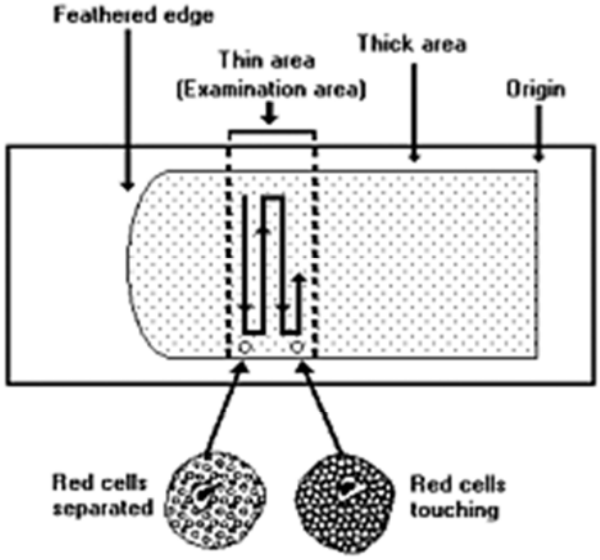
3) WBC morphology, inclusions or toxic granules should be evaluated under this magnification.

OIL immersion or 100x examination of the smear

1) Differential count for 100 WBC is performed ;

Zig - zag manner is adopted to count on the smear and all WBCs are included up to a count of 100 is obtained. (FIG 19)

Figure 19 - Counting in Peripheral smear, a diagrammatic representation



2) RBC's are evaluated for anisocytosis and poikilocytosis, hypochromia or polychromasia and for any inclusions.

3) Platelet estimation along with evaluation of the platelet morphology

Number of platelets are counted in 10 oil immersion fields which is divided by 10 and then the average is multiplied by 15,000[43]. If the number of Nucleated RBC (nRBC) is more than 10 per 100 WBC count, them the following formula is used to calculate the corrected WBC count in mm^3.

$$\text{Corrected WBC count in mm}^3 = \frac{\text{WBC per mm}^3 \text{ x } 100}{100+ \text{ number of nRBC per } 100 \text{ WBC}}$$

This peripheral smear examination is performed in the laboratory as a part of the hematologic lab workup called as CBC - complete blood count.

Normal red blood cells

The red blood cells are described as biconcave cells and has a reddish orange appearance on romanowsky staining. These cells have an average diameter ranging from 7 to 8 um with a central pallor measuring approximately 2 to 3 um. Variations in shape is poikilocytosis and change in size is anisocytosis[43]. The morphology and abnormalities should be examined in the thin portion of the smear where these red cells are slightly separated from each other or sometimes barely touching each other but never overlapping. The thin area selected must also represent about 1/3rd of the entire film. also, RBC's are evaluated for agglutination or rouleaux formation[55]. Agglutination is nothing but aggregates of the red cells into random clusters or masses, whereas rouleaux is the appearance of red cells as stacks of coins during peripheral smear examination[4,43]. Thicker portion of the smear exhibits this rouleaux formation which is artefactual.

Examining platelet morphology

A normal platelet usually measures about 2 to 4 um and has a discoid shape. Elevated platelet count and some morphological changes can be appreciated in a post splenectomy patient and in conditions like idiopathic thrombocytopenic purpura, large platelets are seen[4,43]. Variations in morphology of the platelets are usually seen in diseases infiltrating the boe marrow such as idiopathic myelofibrosis and metastatic infiltrates.

WBC examination on a peripheral smear

The five WBC in their mature form have to be recognized by their individual morphology and leukocytosis have to be noted when the total leukocyte is greater than 10,000 cells / uL. Bacterial infections frequently shoe neutrophilia , sometimes with toxic changes including toxic granulations and inclusions, fungal infections also shows neutrophilia with leukocytosis and sometimes monocytosis is also observed[4,43]. Viral infections predominantly shows lymphocytosis. The most common terminologies we use while reporting a peripheral blood smear are

a) Shift to left - increased neutrophils with an increase in band forms, more immature forms like metamyelocytes and myelocytes can also be noted.

b) Hypersegmentation - Usually the neutrophils have 3 - 4 lobes, five lobes in more than 5 % of the WBC counted or more than 5 lobes is termed as hypersegmentation and most commonly are seen in conditions like megaloblastic anemia, myeloproliferative disorders and even post chemotherapy.

c) Toxic granulations - in bacterial infections, there is increase in number and prominence of the azurophilic granules , sometimes these toxic granulations are also associated with cytoplasmic vacuolations.

d) Dolhe bodies - These are irregularly shaped basophilic stained areas in the cytoplasm seen in infections[43,4]. These are due to Free ribosomes or the rough endoplasmic reticulum and are noted in the neutrophils.

e) Smudge cells - these are predominantly associated with Chronic lymphocytic leukemia, these smudge or basket cells are ruptured cell remnants due to the fragile lymphocytes[6,43].

f) Platelet satellitism - This is an artefact caused by the anticoagulant EDTA, can show artefactul thrombocytopenia.

g) Pelger - Huet anomaly - It is an autosomal dominant condition with bilobed neutrophils

h) May - hegglin anomaly - In this condition, neutrophils shows large, prominent Dolhe like bodies in the cytoplasm[55].

h) Chediak - Higashi syndrome - This is also a rare disorder affecting the neutrophils and show large granules which are due to the abnormal lysosomes.

Materials and Methods

MATERIALS AND METHODS

The study was conducted at Chennai Medical College Hospital, Irungalur, Trichy from September 2014 to September 2016. This was a prospective study and the sample size studied was 120. All adult patients with absolute lymphocytosis diagnosed by cell counter during routine complete blood count tests were included in the study. Relative lymphocytosis diagnosed by the cell counter were also included in the study. The absolute lymphocytosis samples by cell counter were subjected to peripheral smear examination and studied under light microscopy for morphological evaluation. Peripheral smear study was done using Leishman's stain and all the absolute lymphocytosis cases were correlated with clinical history and physical examination. Infection suspected cases were subjected to malarial screening and viral serology. A review of the cases with absolute lymphocytosis were also done to evaluate the lymphocyte count and the morphology after 3 months to one year. The blood samples of patients with persistent lymphocytosis who had undergone medical treatment after a period of 6 months should be subjected to flow cytometry to look for any clonal changes

Clinical profile of patients, biochemical findings and hematological parameters from all the patients were correlated with peripheral blood

smear findings. The brief description of the parameters studied and methods used in detail are elaborated below,

PARAMETERS STUDIED

1. Complete blood count by cell counter; Hematological parameters – Hemoglobin, Total count, Differential count, Platelet count
2. Peripheral blood smear examination for lymphocyte morphology, screening for malarial parasite
3. Clinical parameters – age, gender, diabetes, hypertension, smoking, alcoholism, fever, icterus, joint pain, cough with expectoration, splenomegaly, hepatomegaly, skin examination and lymphadenopathy
4. Biochemical parameters –Viral serology if necessary

INCLUSION CRITERIA

- Adult patients with total lymphocyte count >4000/ul and >3100/ul on cell counter
- Peripheral smear examination showing lymphoid cell count more than 40%

EXCLUSION CRITERIA

- Lymphocytosis on cell counter in patients <12yrs
- Pseudo lymphocytosis on counter

  (after cross checking by peripheral smear examination)

AGE DISTRIBUTION : All patients above 12 years of age

GENDER DISTRIBUTION : Both male and female adult patients are included in the study population.

FEVER : Also known as febrile response or pyrexia is the body temperature above the normal range. The normal range varies between 37.5 and 38.3 °C (99.5 and 100.9 °F). Oral temperature above >37.2 °C (>98.9 °F) in adult males and females is regarded as fever.

ICTERUS: Icterus is the yellowish discoloration of tissue, and seen in sclera of the eye and mucous membranes resulting from the deposition of bilirubin

JOINT PAIN : All adult patients are asked for history of small and large joint pain and are examined for joint tenderness

COUGH WITH EXPECTORATION: All adult patients are asked for history of cough, duration of cough, with or without expectoration and if present with expectoration , its amount and colour.

SMOKER/NON SMOKER : All patients for study are questioned for history of smoking

ALCOHOLIC/ NON ALCOHOLIC: All patients for study are questioned for history of alcohol intake

LYMPH NODE EXAMINATION : All patients are examined for enlarged lymph nodes in bilateral cervical, bilateral axillary, bilateral supraclavicular, bilateral epitrochlear and bilateral inguinal regions by inspection and by palpation method.

HEPATOMEGALY : Both hands are placed side by side flat on the abdomen in the right subcostal region lateral to the rectus , with the fingers pointing towards the ribs, with resistance hands are moved further downwards till the resistance disappears. Gentle pressure is exerted and the patient is asked to breathe in deeply. Hepatomegaly is measured in centimetres palpable below the right costal margin

SPLENOMEGALY : Flat of the left hand is placed over the lowermost ribcage posterolaterally, right hand placed beneath costal margin well out to the left, patient is asked to breathe deeply, simultaneously applying considerable pressure over the left hand . Enlarged spleen is felt as firm swelling below the left costal margin.

SKIN EXAM : Presence or absence of petechiae (red or purple dots) or purpura (red or purple patches) and bruises (ecchymosis) on the skin surface.

HEMATOLOGICAL PARAMETERS

HEMOGLOBIN ESTIMATION

Haemoglobin is going to be estimated by cell counter by the Calorimetric method.

The patients are classified as anaemic when a male has haemoglobin of <13g/dl and female of <12g/dl and the patient is severely anaemic if the haemoglobin value falls below 7g/dl (according to WHO). The same criteria were applied for all the patients.

TOTAL WBC COUNT

Total WBC count and differential counts are estimated by cell counter for all the patients by Electrical Impedance Method.

NORMAL TOTAL WBC COUNT is 4.0 -10.0 x 10^9/l

NORMAL DIFFERENTIAL COUNT is

Neutrophils : 2.0-7.0x10^9(40-80%)

Lymphocytes :1.0-3.0x10^9 (20-40%)

Monocytes : 0.2-1.0x10^9 (2-10%)

Eosinophils : 0.02-0.5x10^9 (1-6%)

Basophils : 0.02-0.1x10^9(<1-2%)

The patient is considered to have leucopenia if the total WBC count is less than 4000 cells/cumm.

The patient is considered to have leucocytosis if the total WBC count is more than 11,500 cells/cumm.

The patient is considered to have lymphocytosis if the differential count of lymphocytes is more than 40%, the patient is considered to have eosinophilia if the differential count of eosinophils is more than 6%, basophilia if differential count of basophils are more than 2%, monocytosis if differential count of monocytes are more than 10%, neutropenia and neutrophilia if differential count of neutrophils are less than 40% and more than 80% respectively.

ABSOLUTE LYMPHOCYTOSIS AND RELATIVE LYMPHOCYTOSIS

ABSOLUTE LYMPHOCYTOSIS: Absolute lymphocytosis is present when a patient's blood sample shows absolute lymphocyte count more than 4000/microlitre. Absolute lymphocyte count (ALC) is calculated by multiplying the percentage of lymphocytes counted in peripheral smear/cell counter and multiplied by the total WBC count.

RELATIVE LYMPHOCYTOSIS: The patient is said to have relative lymphocytosis when there are >40% lymphocytes in the peripheral smear/ cell counter values with a normal absolute lymphocyte count (< 4000/microlitre).

PLATELET COUNT: Platelet count is estimated by cell counter for all the patients by Electrical Impedance method.

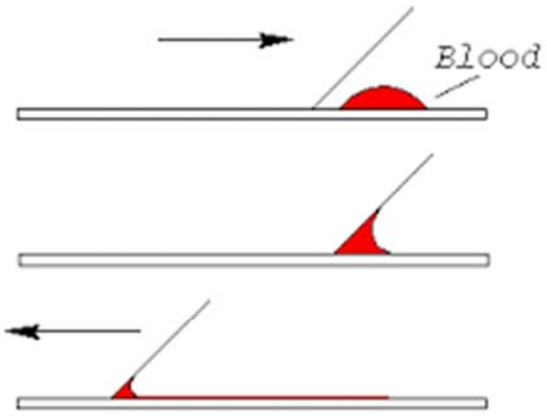
Normal platelet count is 1,50,000/cumm and the patient is considered thrombocytopenic when the patient platelet count falls below less than 1,50,000/cumm.

PERIPHERAL SMEAR: All the patients are subjected for peripheral blood smear examination, prepared by Leishman's staining procedure and studied under light microscope in oil immersion .

PERIPHERAL BLOOD SMEAR PROCEDURE

Blood smears are prepared from either a fresh blood sample or from EDTA (ethylenediaminetetraacetic acid) sample. The slides selected for a blood smear should measure 75 x 25 mm and thickness should be about 1 mm. A spreader slide is prepared by selecting a glass slide with one end smooth and a width of 18 mm. This is prepared by using a glass cutter and breaking off one corner of the slide and smoothening the end. A drop of blood is placed in the centre line about 1 cm away from one end of the slide. Without any delay, the selected spreader slide is placed in front of the drop at an angle of 30^0 to the slide and is moved back to be in contact with the placed drop. The drop spreads rapidly along the line in contact. with a steady movement of the hand, the blood is spread along the slide without taking off the hand until the last trace of blood is spread on the

slide. The total length of the blood film should be measuring about 3 cm and the must finish about 1 cm before the end of the slide. Ideally, thickness of the film is usually adequate when there is some overlap of the red blood cells almost throughout the slide. These films are allowed to dry in air.



STAINING OF BLOOD SMEAR

Romanowsky stains are the stains which are used universally for routine staining of blood films. Originally they were a combination of polychrome methylene blue and eosin. Nowadays, azure B is also incorporated. Of the romanowsky stains, The giemsa stain is the most complex and the Jenner is the simplest stain. Leishman stain is an intermediate stain. The mechanism of these dyes are that the azure B binds to the anionic molecules whereas eosin Y binds to the cationic sites on the proteins, thereby the nucleic acids and the proteins of the cell nucleus and the cytoplasm takes up the basic dye Azure B whereas the basic grouping

of the hemoglobin molecules has affinity to acidic dye is stained by eosin. The various stains of the romanowsky dyes are May- Grunwald stain, Jenner stain, Giemsa and Leishman stain. Buffered water is prepared by taking 50 ml of 66 mmol/l Sorensen's phosphate buffer in a PH of about 6.8 with one litre of water.

Staining with Leishman stain - The air dried film is flooded with the stain for two minutes and double the amount of water for about five to seven minutes. This slide is washed under a stream of buffered water till it acquires a pinkish tinge for about two minutes. this is followed by wiping the slide on the back side and it is kept upright to dry. The colours taken up by the various structures stained with Romanowsky stain are purple colour by the nuclear chromatin, light blue colour by the nucleoli, cytoplasm of the neutrophil is pink orange, of the lymphocyte and basophil is blue, granules in basophil are purple black, those of eosinophils are red- orange, of neutrophils are purple and the platelets also stain purple colour.

Relevant cases were subjected to biochemical tests like C -Reactive protein and LDH.

To summarize, for all patients with absolute lymphocytosis, a detailed clinical history and clinical examination was sort followed by peripheral smear examination to study the morphology of lymphocytes. Relevant hematological and biochemical parameters were also studied. In

the absence of a neoplastic process, underlying cause was treated and the patients were called in for a review after 3 to 6 months and subjected to complete blood count and peripheral smear examination.

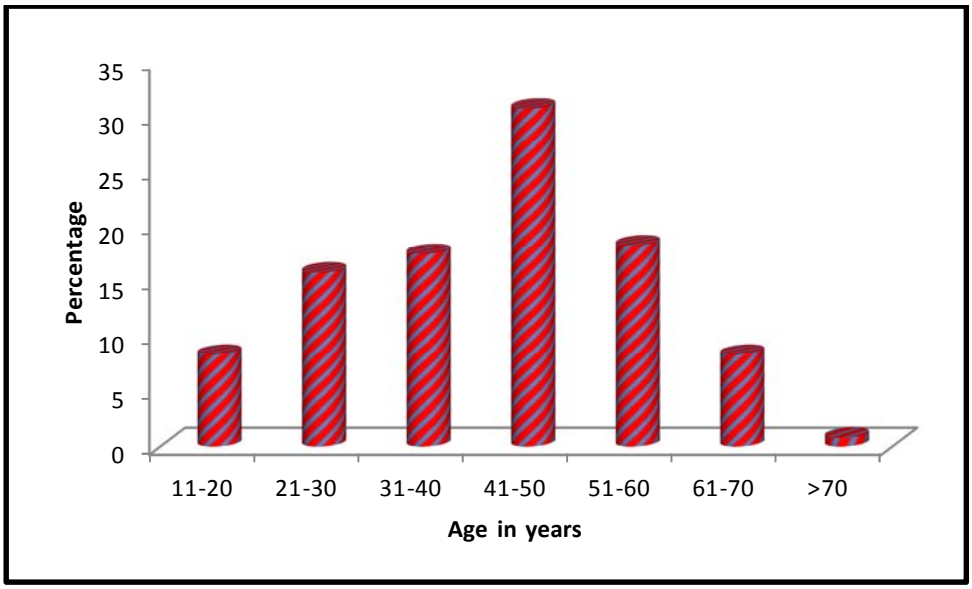
Results

RESULTS

This study was conducted to evaluate the morphology of lymphocytes in absolute lymphocytosis in adults patients, correlate the results with clinical, hematological and biochemical parameters, and to determine the cut off range of lymphocytosis for morphological evaluation. A total number of 120 cases were followed up over a period of 2 years, out of which 80 patients showed absolute lymphocyte count more than 4000/ul. Remaining 40 patients showed an absolute lymphocyte count more than 3000/ul. All the patients were evaluated after a period of three to six months to assess the morphology of the lymphocytes.

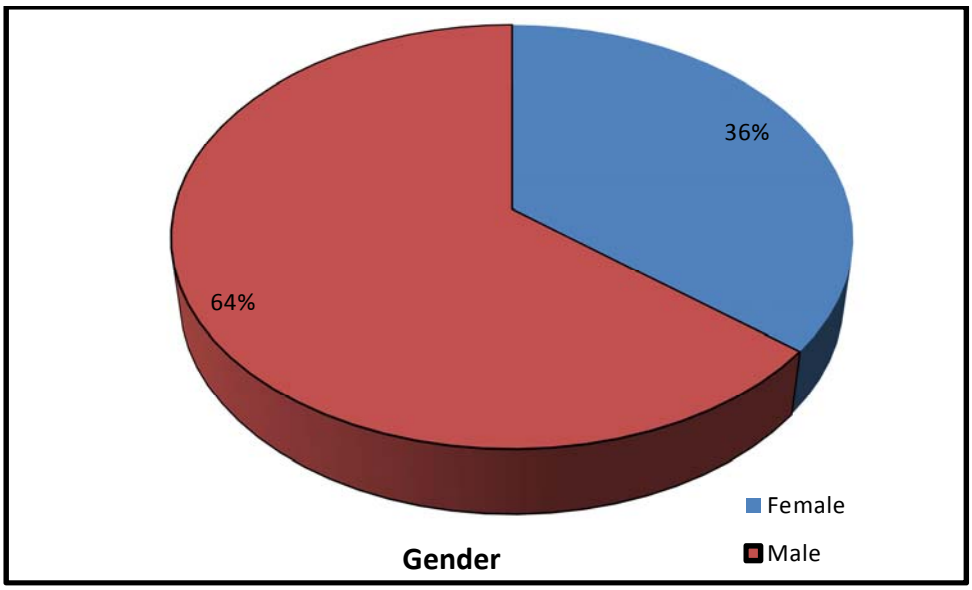
Cases evaluated are in the age group between 13 to 80 years. Majority of patients in the study group are between 41 to 50 years constituting 30.08% of the total number of the cases. The youngest patient in this study was 13 years and the oldest patient was 80 years.[**Chart 1**]

Chart 1- Age wise distribution of lymphocytosis



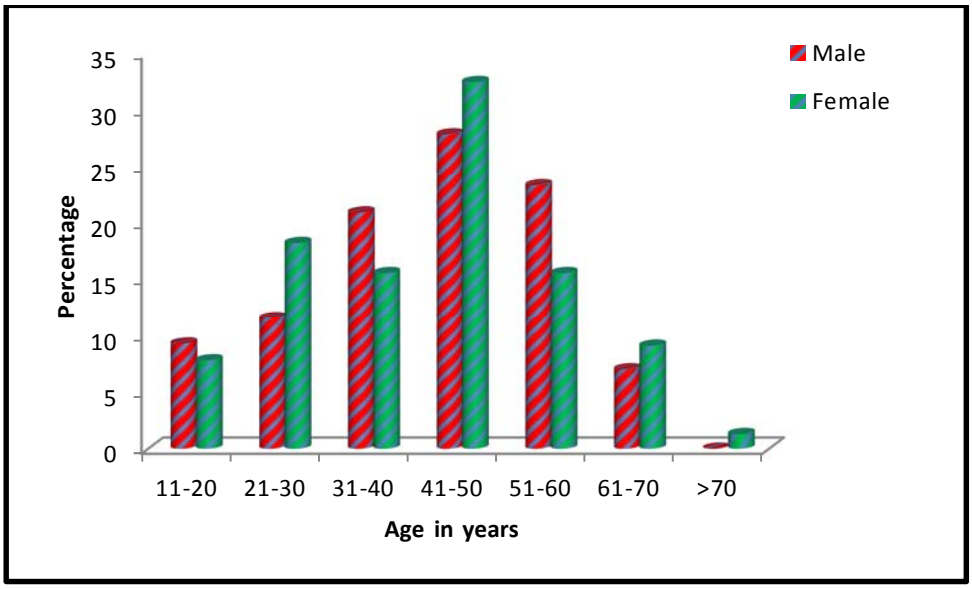
Out of the 120 cases studied, 35.8 % (43 patients) were females and 64.2% (77 patients) were males (**Chart- 2)**

Chart 2- Sex distribution of the cases studied



Maximum number of patients were between 21 to 60 years constituting 99 cases. Out of the them, 36 patients are females and 63 of the patients are males. **(Chart - 3)**

Chart 3- Age wise sex distribution



Out of the total 120 patients studied, 30 patients had an history of fever, 23 patients had an acute stress induced history (acute abdominal pain, RTA/ trauma, chest pain/discomfort and seizure episode), 7 of them showed an acute psychiatric illness and 7 of them showed skin related lesions. Remaining of the patients included master health check up patients, patients posted for surgery from various departments and patients with symptoms like headache/ cough and urinary tract infection. **(Table- 3)**

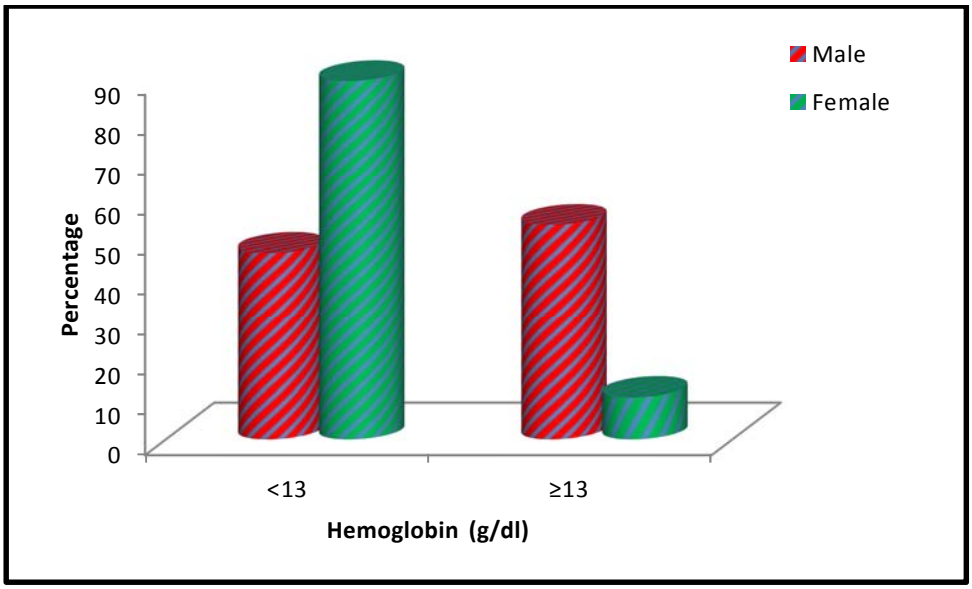
Table 3 - Distribution of complaints

Complaints	Gender		Total
	Female	Male	
Fever	8(18.6%)	22(28.6%)	30(25%)
Cough	10(23.3%)	6(7.8%)	16(13.3%)
Abdomial pain	2(4.7%)	6(7.8%)	8(6.7%)
Psycosis (psychiatric illness)	1(2.3%)	6(7.8%)	7(5.8%)
Generalised itching	1(2.3%)	4(5.2%)	5(4.2%)
Master health check up	2(4.7%)	3(3.9%)	5(4.2%)
Trauma	1(2.3%)	4(5.2%)	5(4.2%)
RTA	3(7%)	1(1.3%)	4(3.3%)
Chest pain (chest discomfort)	2(4.7%)	1(1.3%)	3(2.5%)
Headache	1(2.3%)	2(2.6%)	3(2.5%)
Bleeding P/V	1(2.3%)	1(1.3%)	2(1.7%)
Burns	1(2.3%)	1(1.3%)	2(1.7%)
Discharge P/V	0(0%)	2(2.6%)	2(1.7%)
Leg pain	2(4.7%)	0(0%)	2(1.7%)
Throat pain	1(2.3%)	1(1.3%)	2(1.7%)
Uncontrolled DM	1(2.3%)	1(1.3%)	2(1.7%)
Breathlessness, myalgia	0(0%)	1(1.3%)	1(0.8%)
Burning micturition, white discharge P/V	0(0%)	1(1.3%)	1(0.8%)
Chest discomfort	0(0%)	1(1.3%)	1(0.8%)
Diarrhoea, abdominal pain, vomiting	1(2.3%)	0(0%)	1(0.8%)
Dyspnea, cough	0(0%)	1(1.3%)	1(0.8%)
Ear pain, discharge	0(0%)	1(1.3%)	1(0.8%)
Fibroadenoma breast	0(0%)	1(1.3%)	1(0.8%)
Giddiness	0(0%)	1(1.3%)	1(0.8%)
H/o tiredness	1(2.3%)	0(0%)	1(0.8%)
HIV	0(0%)	1(1.3%)	1(0.8%)
Mass P/V	0(0%)	1(1.3%)	1(0.8%)
Pain, multiple joints	1(2.3%)	0(0%)	1(0.8%)
Prolapse uterus	0(0%)	1(1.3%)	1(0.8%)
Proptosis	1(2.3%)	0(0%)	1(0.8%)

Right inguinal hernia	1(2.3%)	0(0%)	1(0.8%)
Scalp abscess, fever	0(0%)	1(1.3%)	1(0.8%)
Skin lesions(utricaria, generalised itching)	0(0%)	1(1.3%)	1(0.8%)
Seizures (recurrent seizures)	2(4.7%)	1(1.3%)	3(2.5%)
Swelling right popliteal fossa, burning micturition	0(0%)	1(1.3%)	1(0.8%)
Tiredness/weakness	0(0%)	1(1.3%)	1(0.8%)
Tonsillitis	0(0%)	1(1.3%)	1(0.8%)
Tuberculosis	1(2.3%)	0(0%)	1(0.8%)
Utricaria	0(0%)	1(1.3%)	1(0.8%)
Total	43(100%)	77(100%)	120(100%)

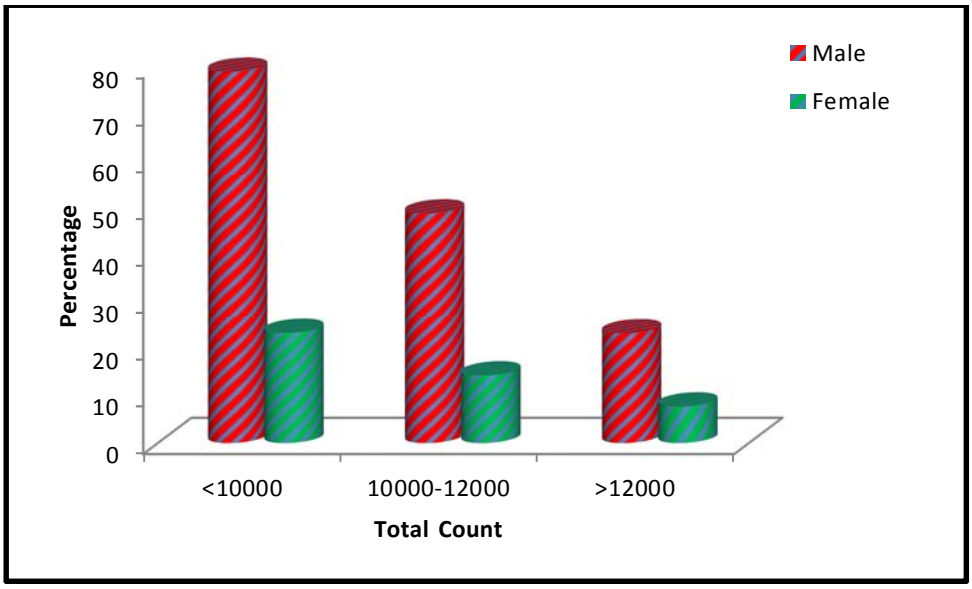
During routine hematological investigations, out of the total 120 patients studied, hemoglobin predominantly was less than 13g/dl in about 89 patients and only 31 patients had a hemoglobin count more than 13g/dl.**(Chart -4)**

Chart 4- Sex wise distribution of hemoglobin (g/dl)



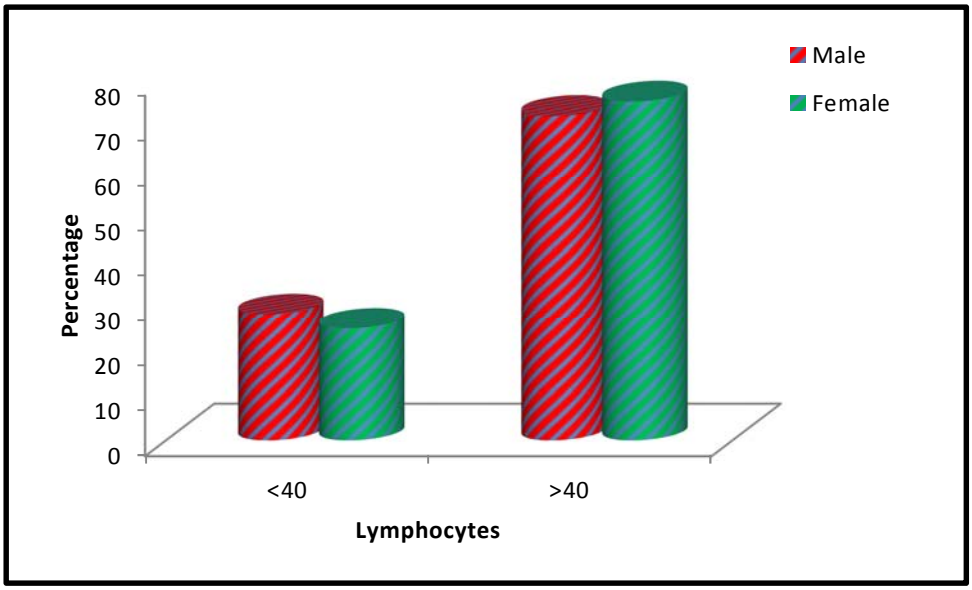
Out the 120 patients under this study, routine hematological investigations revealed 52 patients to have a total count less than 10,000/ul followed by 32 patients showing a total count between 10,000/ul to 12,000/ul and an increased total count (leukocytosis) in 16 patients**. (Chart -5)**

Chart 5- Distribution of total count in the study population



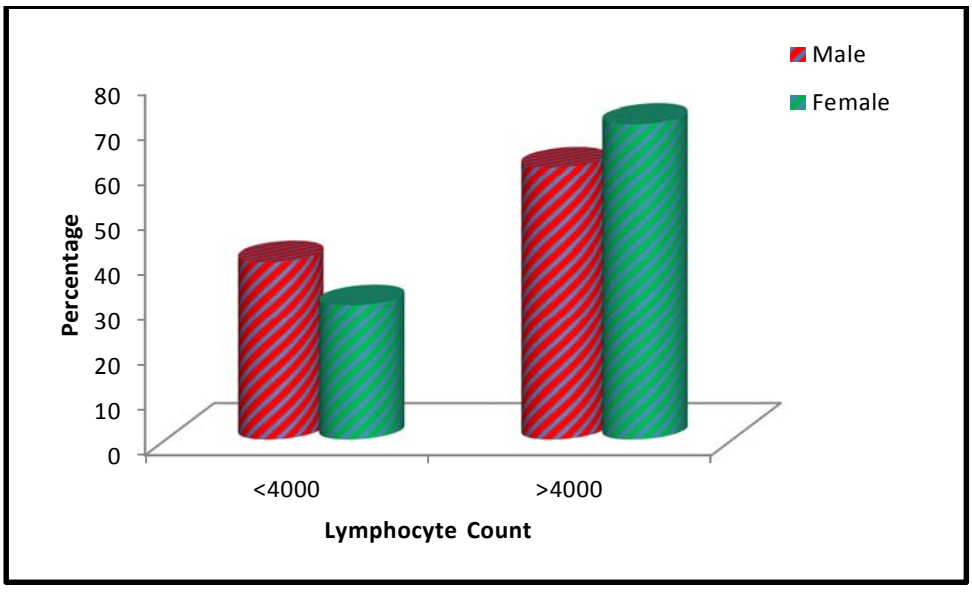
Routine hematological investigation by the cell counter of all the 120 patients showed that 31 patients had a lymphocyte count less than 40% and majority of them, about 89 patients had a lymphocyte count more than 40%.(**Chart -6**)

Chart 6 - Lymphocyte percentage distribution in the study population



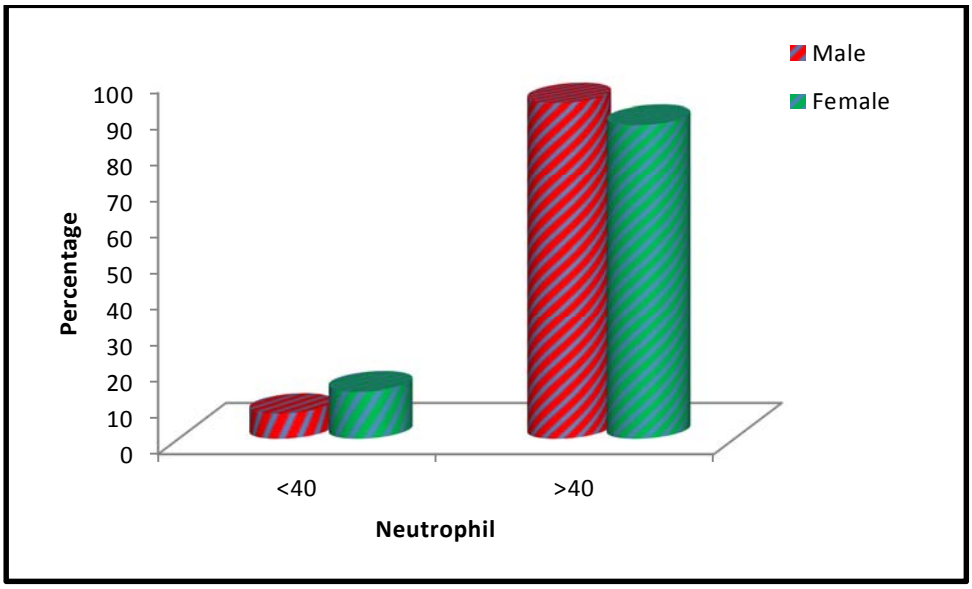
Absolute lymphocyte count calculated was more than 4000/ul in 80 patients and the absolute lymphocyte count in the remaining 40 patients was less than 4000/ul. (**Chart -7**)

Chart 7- Calculated ALC distribution in the study population



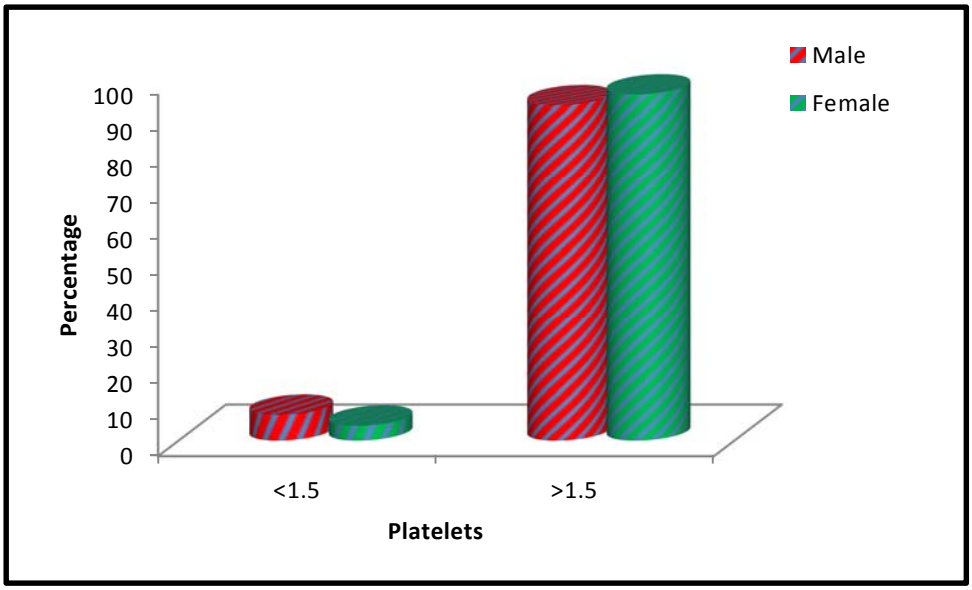
Neutrophil percentage calculated by the automated cell counter was more than 40% in 90% of the patients studied and only 10% of the patients showed a neutrophil percentage less than 40%. (**Chart 8**)

Chart 8 - Neutrophil percentage distribution



Out of the 120 patients studied, only 6 patients showed a decrease in platelet count less than 1.5 lakhs/cumm.(**Chart -9**)

Chart 9- Platelet distribution in the study population



Other hematological/ clinical/ biochemical tests which were performed with suspicion of various other illness in relation to the clinical history and symptoms to the patients were mantoux test for patients with a previous history of tuberculosis or present clinical symptoms like cough with expectoration or a suspicion in chest x-ray; dengue serology - including both IgG and IgM; malaria card test; urine routine for pus cells in patients with lower abdominal pain and burning micturition; serology for HIV/ HBSAg/HCV in suspected cases and an ESR study.

Out of 120 patients, one of these other tests were performed only on 16 patients, rest of the patients did not show relevance in doing further tests.

During routine peripheral smear screening of all the 120 patients, 1 patient had malarial parasite positivity in peripheral smear for whom malaria card test also showed positivity. (**Chart 10, Chart -11**)

Chart 10 - Distribution of other tests done

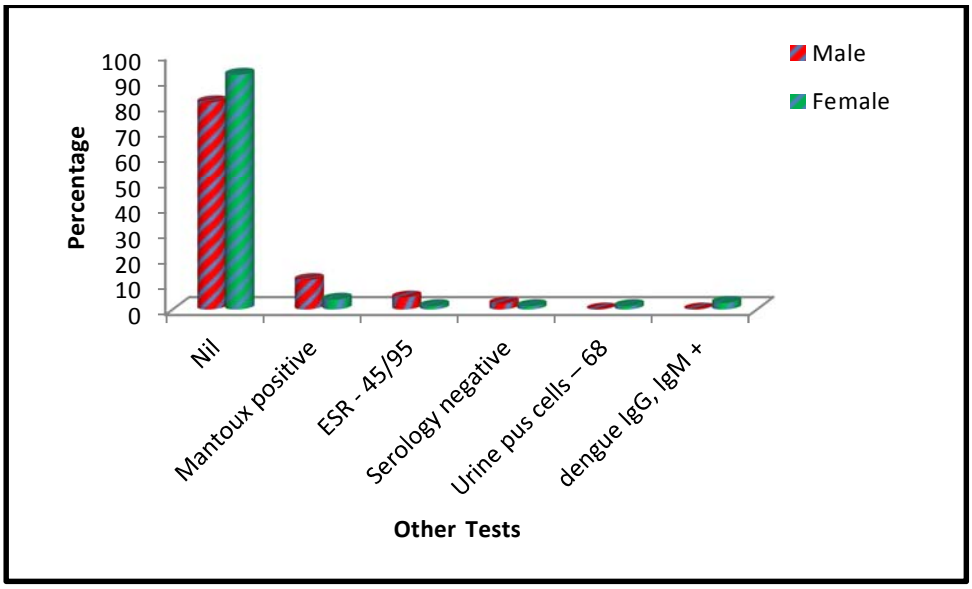
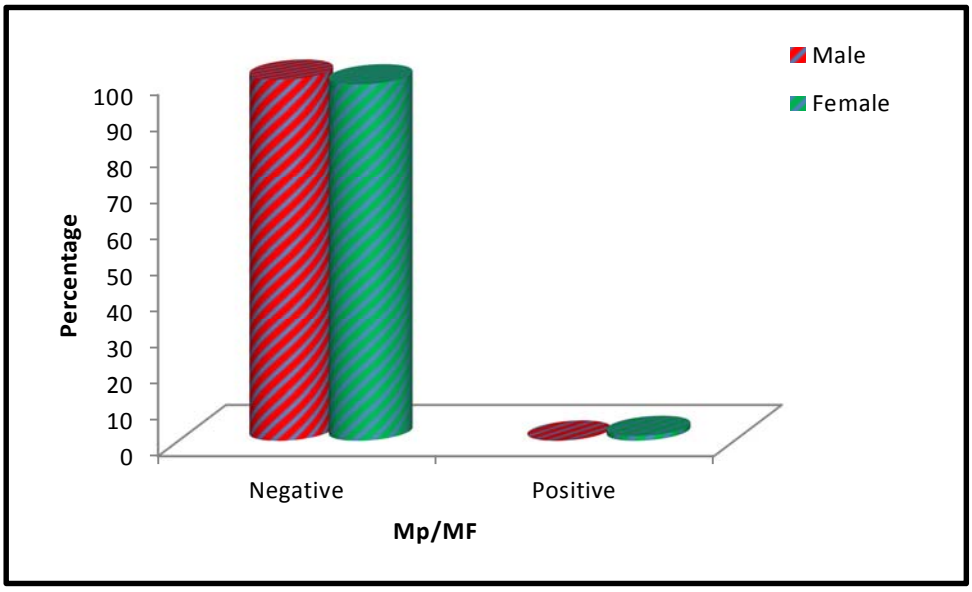
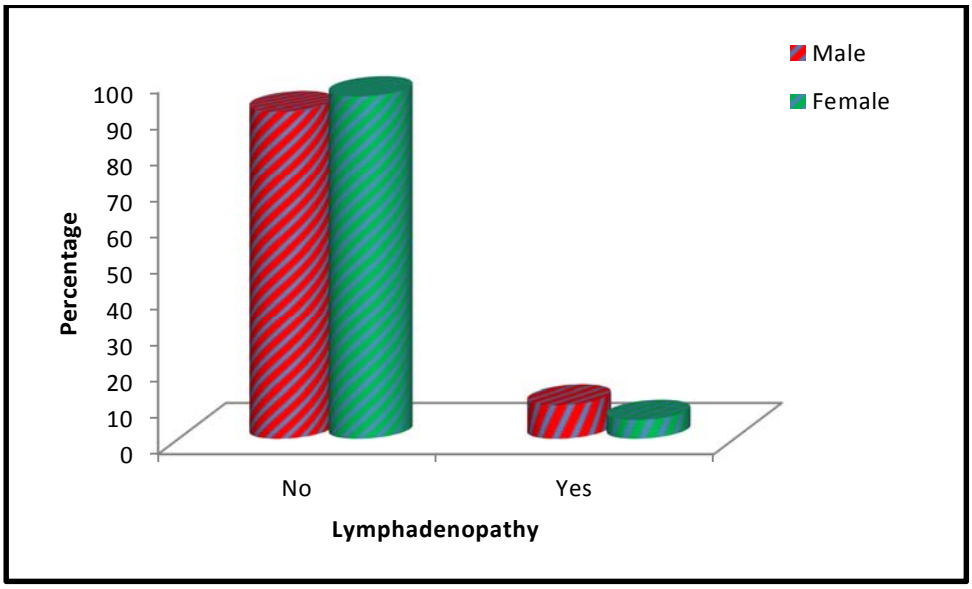


Chart 11- Malarial parasite positivity distribution in the study population



All the 120 patients were clinically examined for lymphadenopathy, only 8 patients showed lymphadenopathy involving either cervical/ axillary or inguinal nodes. (**Chart -12)**

Chart 12 - Lymphadenopthy in the study population



Out of the 8 patients, 5 patients had cervical node enlargement, 2 had axillary node involvement and 1 patient showed inguinal node enlargement. (**Table-4**)

Table 4 - Lymphadenopathy distribution

Lymphadenopathy	Gender		Total (n=120)
	Female (n=43)	Male (n=77)	
No	39(90.7%)	73(94.8%)	112(93.3 %)
Yes	4(9.3%)	4(5.2%)	8(6.7%)
• Cervical lymph node +	3(7%)	2(2.6%)	5(4.2%)
• Axillary nodes +	0(0%)	2(2.6%)	2(1.7%)
• right inguinal positive 1.5 x 1.5 cm	1(2.3%)	0(0%)	1(0.8%)

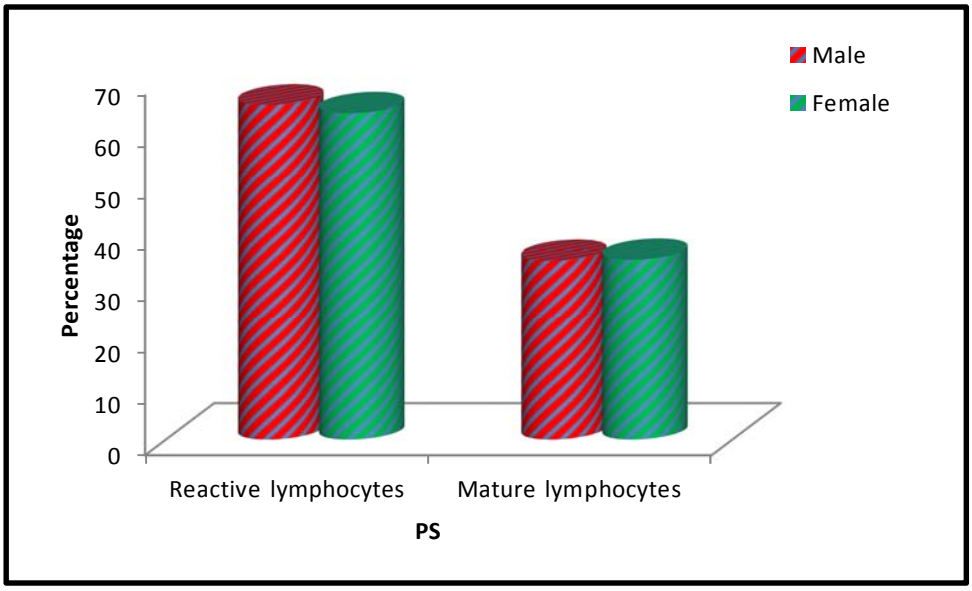
Only one patient in the study population showed atypical lymphocytes and blasts in peripheral smear and she was subjected to bone marrow evaluation.(**Table-5**)

Table 5- Distribution of bone marrow examination

BM	Gender		Total
	Female	Male	
No	42(97.7%)	77(100%)	119(99.2%)
Yes	1(2.3%)	0(0%)	1(0.8%)
Total	43(100%)	77(100%)	120(100%)

Out of the 120 peripheral smears examined, 77 of them showed a reactive/ atypical lymphocyte morphology and 43 of them showed a mature lymphocyte morphology.(**Chart -13**)

Chart 13 - Distribution of reactive lymphocytes and mature lymphocytes in the study population



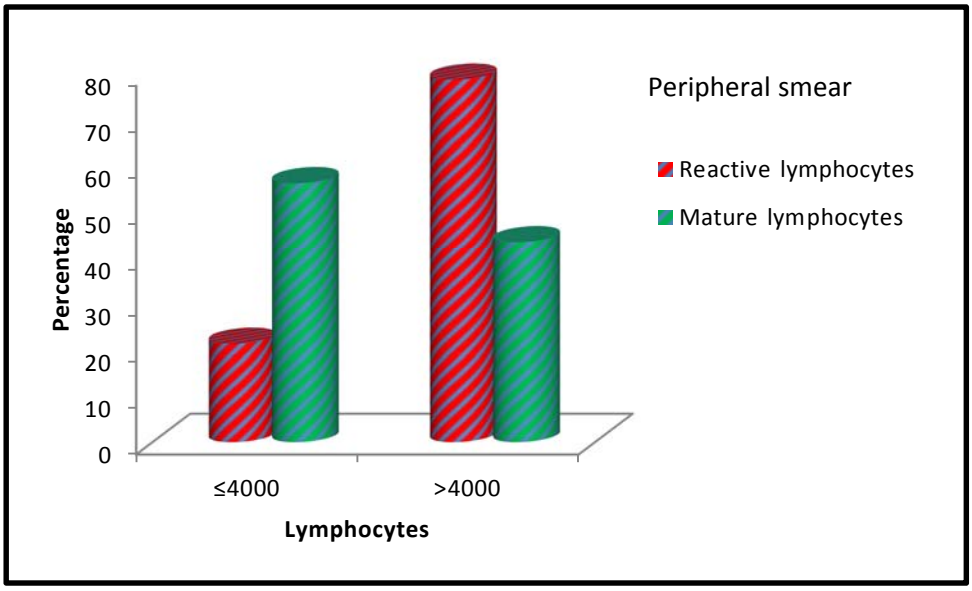
Out of the total reactive lymphocyte morphology, majority of about 80% of the patients have a absolute lymphocyte count more than 4000/ul. **(Chart -14)(Table-6)**

Table 6 - Comparison of distribution of lymphocyte morphology with ALC

Lymphocytes	Peripheral smear		Total
	Reactive lymphocytes	Mature lymphocytes	
≤4000	17(21.3%)	23(56.4%)	40(35%)
>4000	68(78.7%)	12(43.6%)	80(65%)
Total	85(100%)	35(100%)	120(100%)

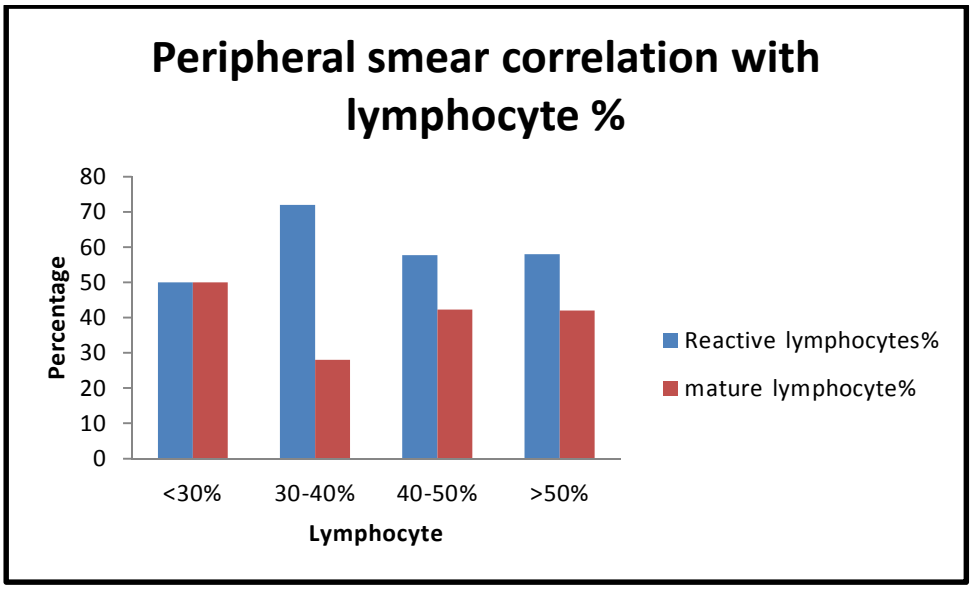
P<0.001, significant, Chi-Square test

Chart 14- Comparison of distribution of lymphocyte morphology with ALC



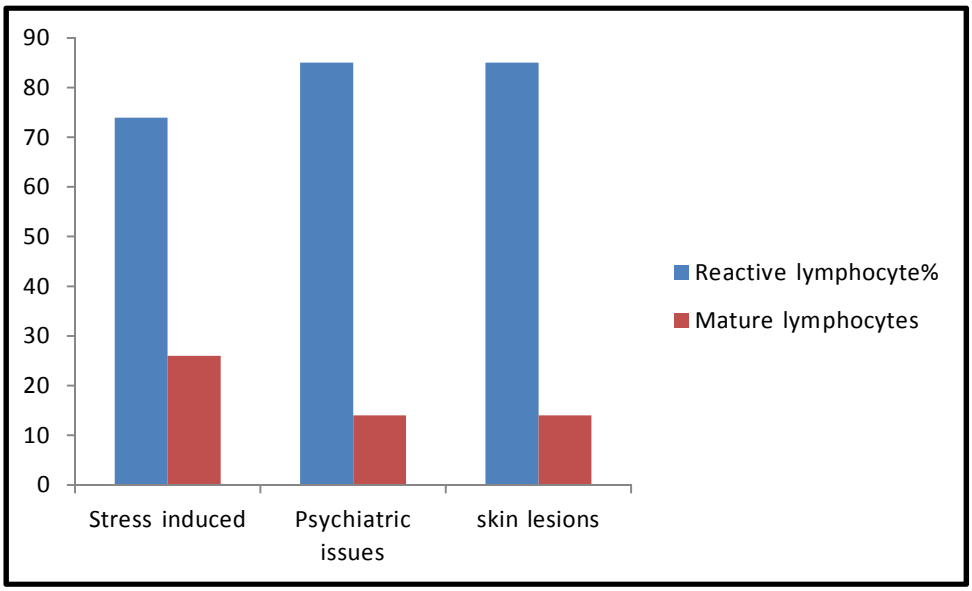
Patients with a lymphocyte percentage of 30 - 40 % were 25 in number, out of which, 18 of them showed a reactive/atypical lymphocyte morphology compared to only 7 of the patients showing a normal mature morphology of the lymphocytes. Similarly, 52 patients had a lymphocyte percentage between 40 to 50%, out of which 30 patients showed reactive lymphocyte morphology and 22 of them showed a mature morphology of lymphocyte. Out of the 21 patients with a lymphocyte percentage more than 50%, 12 of them showed a reactive lymphocyte morphology compared to only 9 of them showing a mature morphology of the lymphocytes.(**Chart -15**)

Chart 15- Peripheral smear correlation with lymphocyte percentage



Out of the 23 patients with stress induced history, 17 of them showed reactive lymphocytes, of the 7 patients with skin related problems, 6 showed reactive lymphocyte morphology and 6 out of 7 patients with psychiatric issues showed reactive lymphocyte morphology.(**Chart -16)**

Chart 16- Distribution of reactive and mature lymphocytes in association with clinical symptoms



Out of the major complaints studied , patients who had an history of stress were 23, out of which 18 had an absolute lymphocyte count more than 4000/ul, all patients with history of skin associated lesions had absolute lymphocyte count more than 4000/ul and of the 7 patients with an acute episode of psychiatric problem, 5 of them showed absolute lymphocyte count more than 4000/ul.(**Table-7**)

Table 7 - ALC distribution in association with patient complaints

Complaints	**Lymphocytes**		**Total**
	≤4000	**>4000**	
Stress Induced	5(71.4%)	18(69.2%)	23(62.1%)
Skin lesions	(0%)	7(11.5%)	7(18.9%)
Psychiatric problems	2(28.6%)	5(19.2%)	7(18.9%)
Total	7(100%)	30(100%)	37(100%)

Out of 120 patients, 6 patients with complaints of fever were subjected to C- Reactive protein test and 3 patients with cardiac symptoms were subjected to LDH. (**Table - 8**)

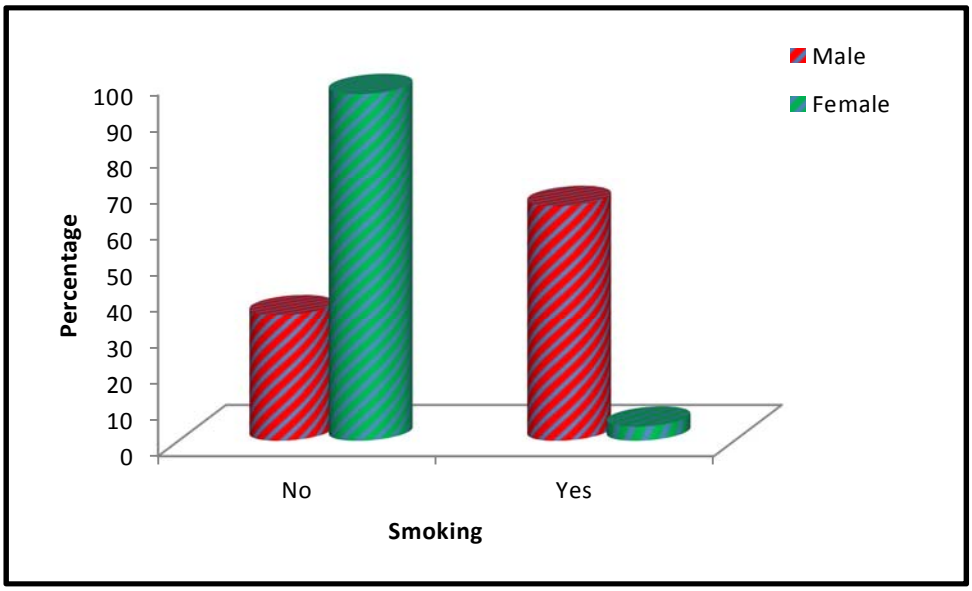
Table -8 Distribution of biochemical parameters studied

Tests	**No. of patients**
CRP Positive	2
CRP negative	4
LDH elevated	1
LDH- not elevated	2

Both the patients with positive C reactive protein did not show any reactive lymphocytes in the peripheral smear examination. One patient with elevated levels of LDH, had heart failure symptoms and showed reactive lymphocytes in peripheral smear along with an absolute count more than 4000/ul.

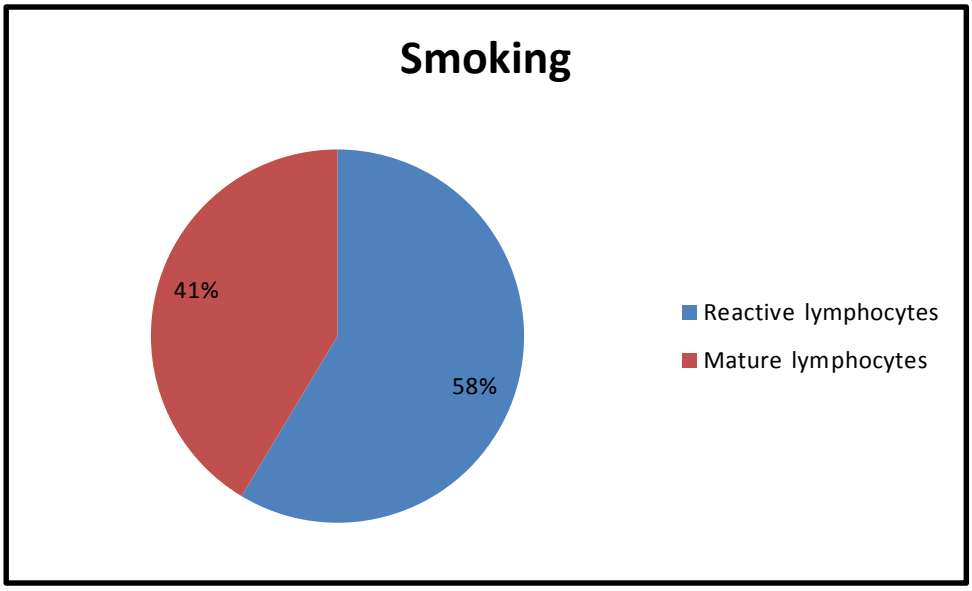
Out of the 120 patients, a total of 31 patients had history of smoking/tobacco chewing.(**Chart -17**)

Chart 17- Distribution of smoking history in the study population



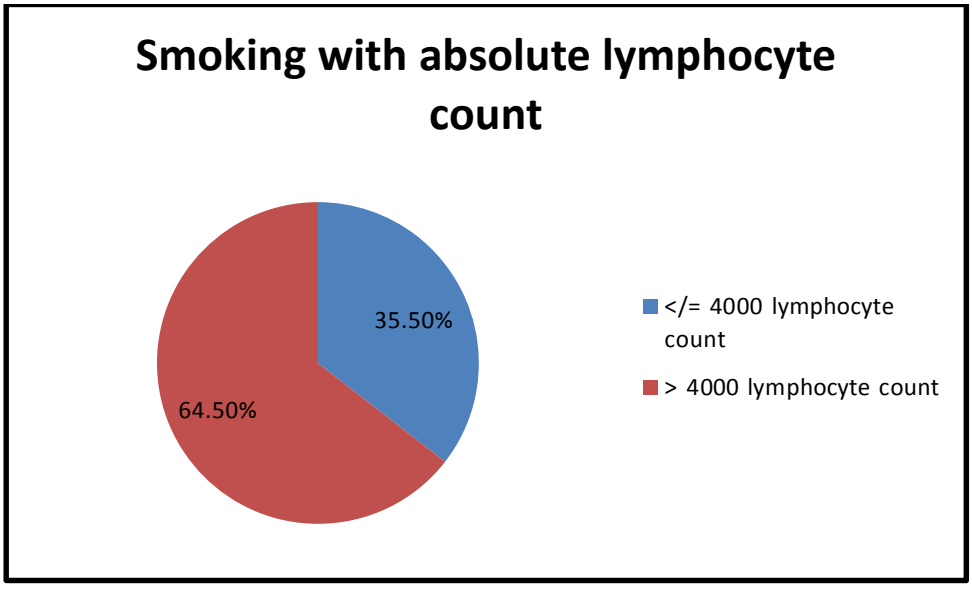
Out of the 31 patients with positive smoking history, 18(58%)of them showed reactive/ atypical lymphocyte morphology and 13(41%) of them showed mature lymphocytes.(**Chart -18**)

Chart 18 - Distribution of reactive and mature lymphocytes in patients with smoking history



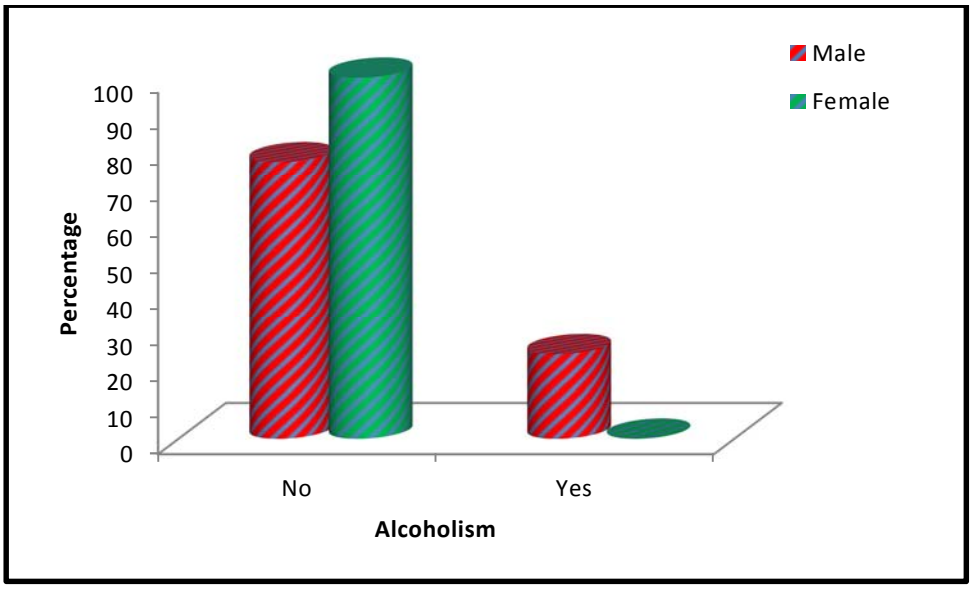
Out of the 31 cases with smoking history, 20 (64.5%) of them had an absolute count more than 4000/ul and the rest 11 (35.5%) patients had ALC less than 4000/ul.(**Chart -19)**

Chart 19 - Comparison of ALC with smoking history



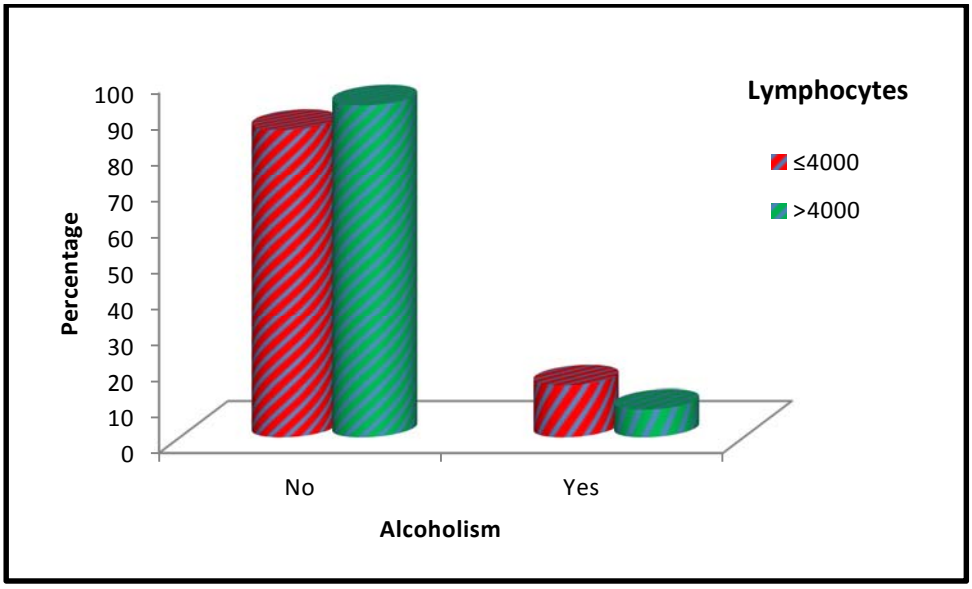
Out of the 120 patients assessed with absolute lymphocytosis, 10 of the patients had positive alcoholism history.(**Chart -20**)

Chart 20 - Prevalence of alcoholism in the study population



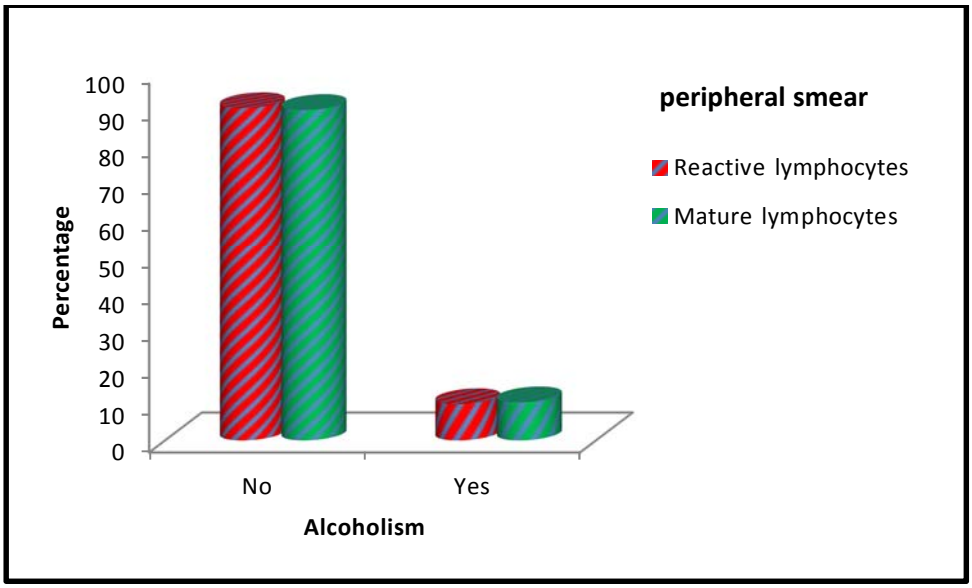
Out of the 10 patients with alcoholism history, 5 patients had an absolute lymphocyte more than 4000/ul and other 5 patients had an absolute lymphocyte count less than 4000/ul. (**Chart -21**)

Chart 21 - Comparison of ALC and alcoholism



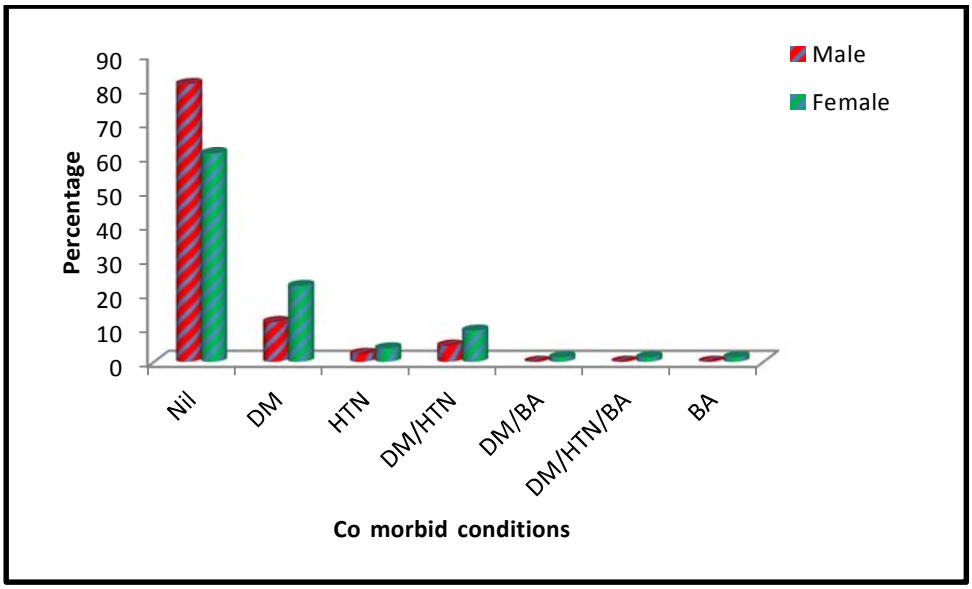
Of the 10 patients with alcoholism history, only 4 of them showed a normal mature morphology of lymphocytes, other 6 patients showed reactive lymphocyte morphology.(**Chart -22**)

Chart 22- Distribution of lymphocyte morphology in alcoholics



Out of the total 120 cases studied, about 70% of the population did not have any co-morbid conditions associated. Rest of the patient population showed a major association with diabetes.(**Chart -23**)

Chart 23 - Association of study population with co morbid conditions

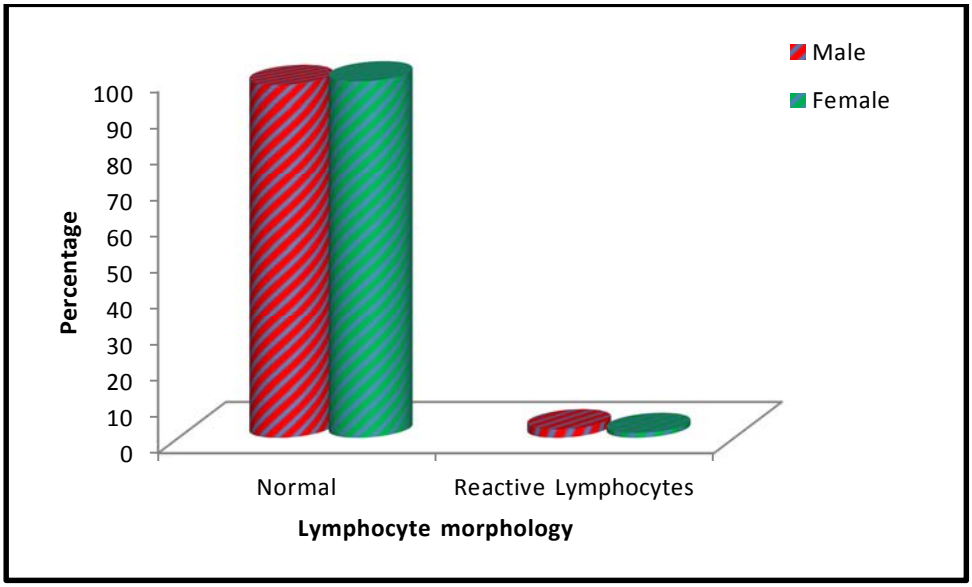


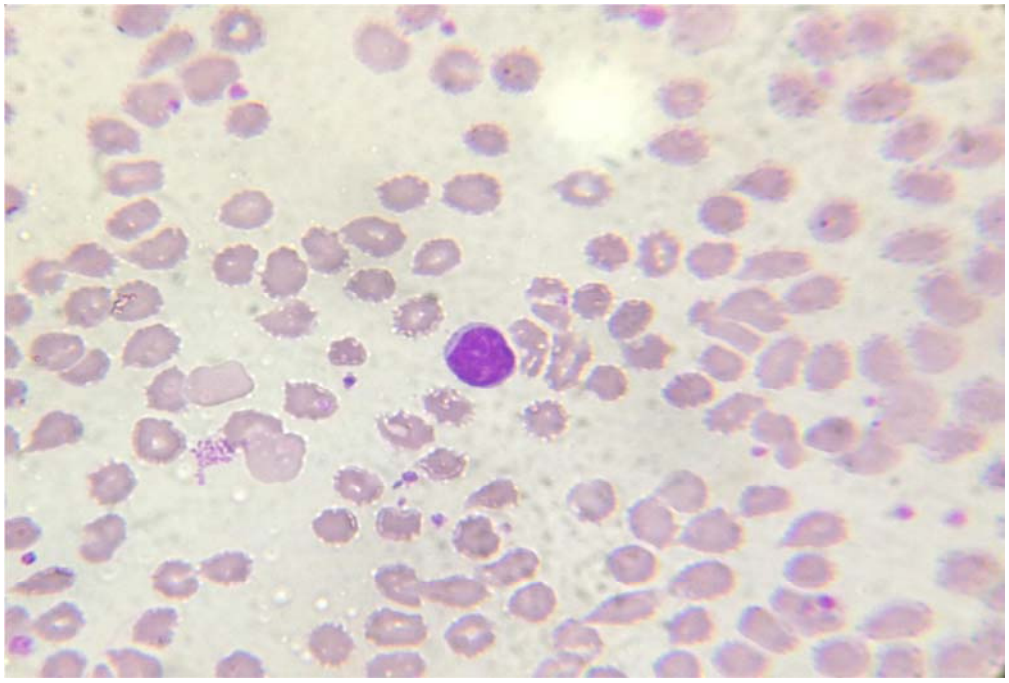
Of all the patients who were reviewed with a repeat peripheral smear examination after a period of three to six months, 98 % of the patients after treatment had normal mature lymphocytes. Only two of them showed persistence of the reactive lymphocytes in their blood. (**Table-9**)(**Chart - 24**)

Table 9- Distribution of lymphocytes in review peripheral smear

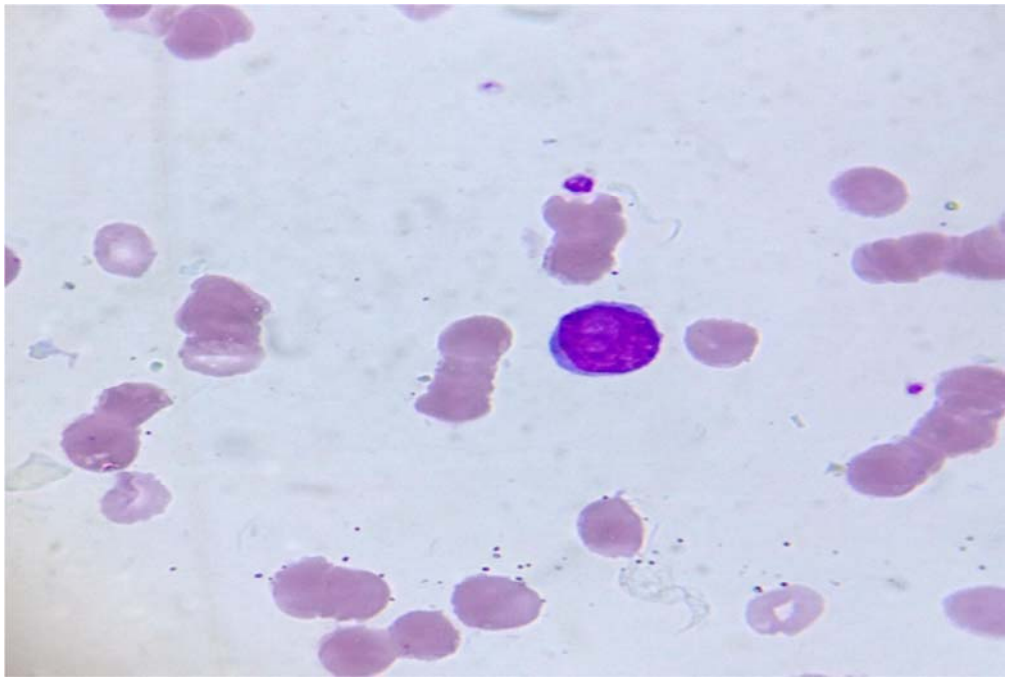
Lymphocyte morphology	**Gender**		**Total**
	Female	**Male**	
Normal	42(97.7%)	76(98.7%)	118(98.3%)
Reactive Lymphocytes	1(2.3%)	1(1.3%)	2(1.7%)
Total	43(100%)	77(100%)	120(100%)

Chart 24 - Distribution of lymphocytes in review peripheral smear

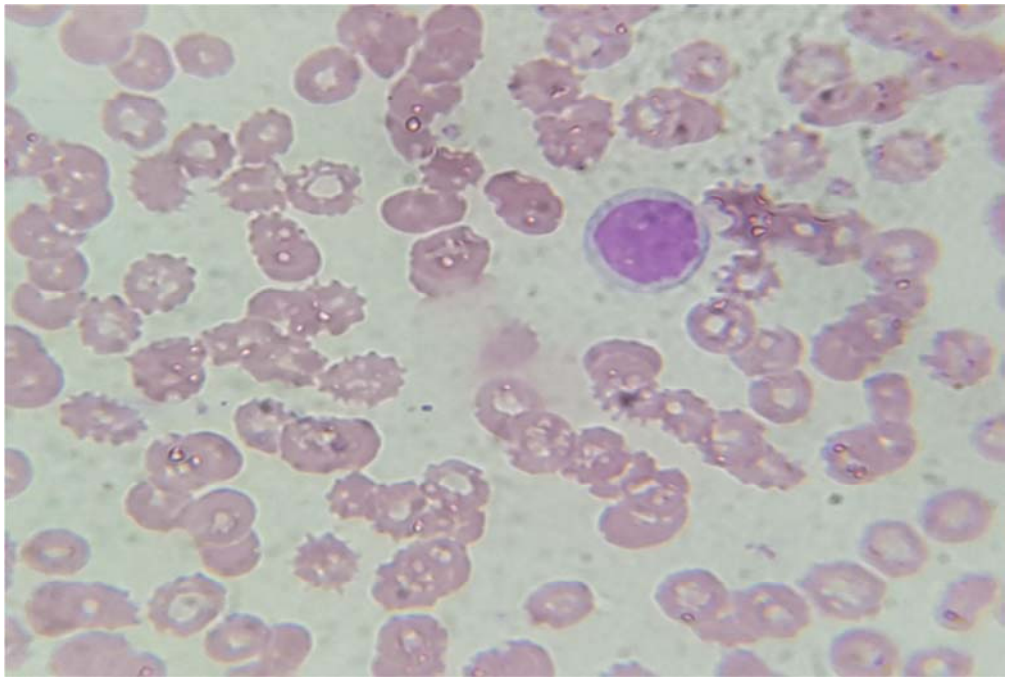




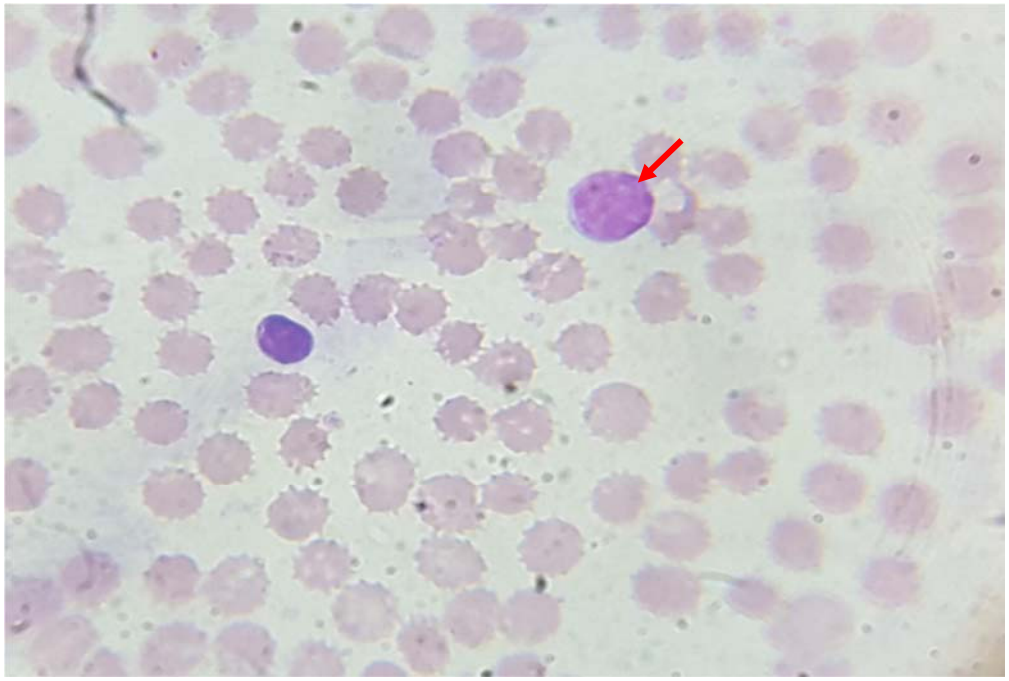
Colour Plate 1 : Peripheral smear showing mature lymphocyte in Leishman stain (100x)



Colour plate 2 : Peripheral smear showing large lymphocyte with platelets in Leishman stain (100x)

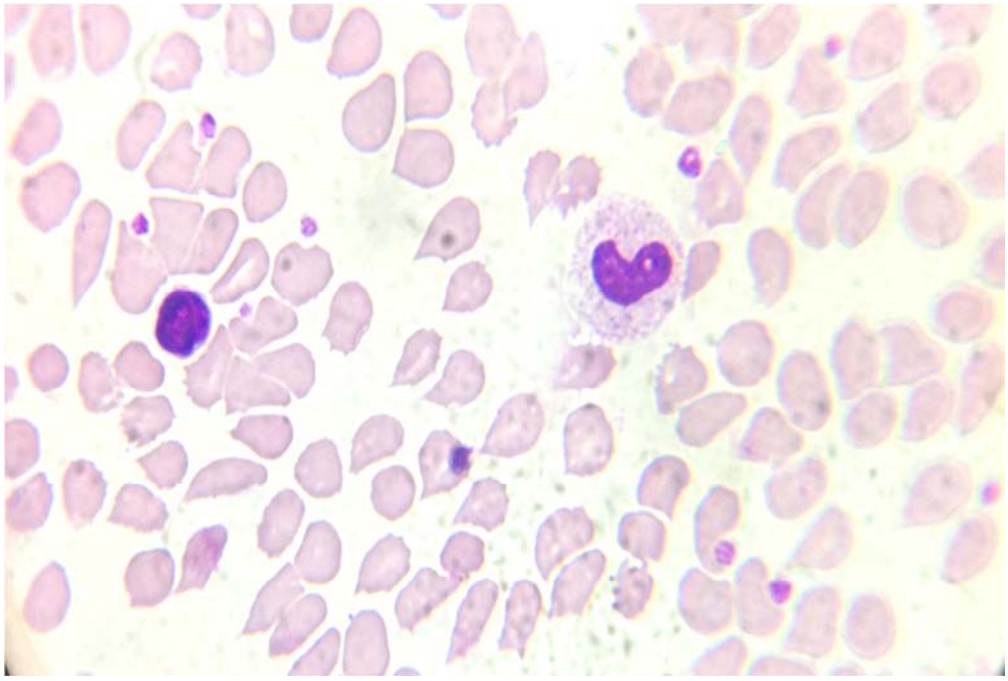


Colour Plate 3 : Peripheral smear showing reactive lymphocyte in Leishman stain (100x)

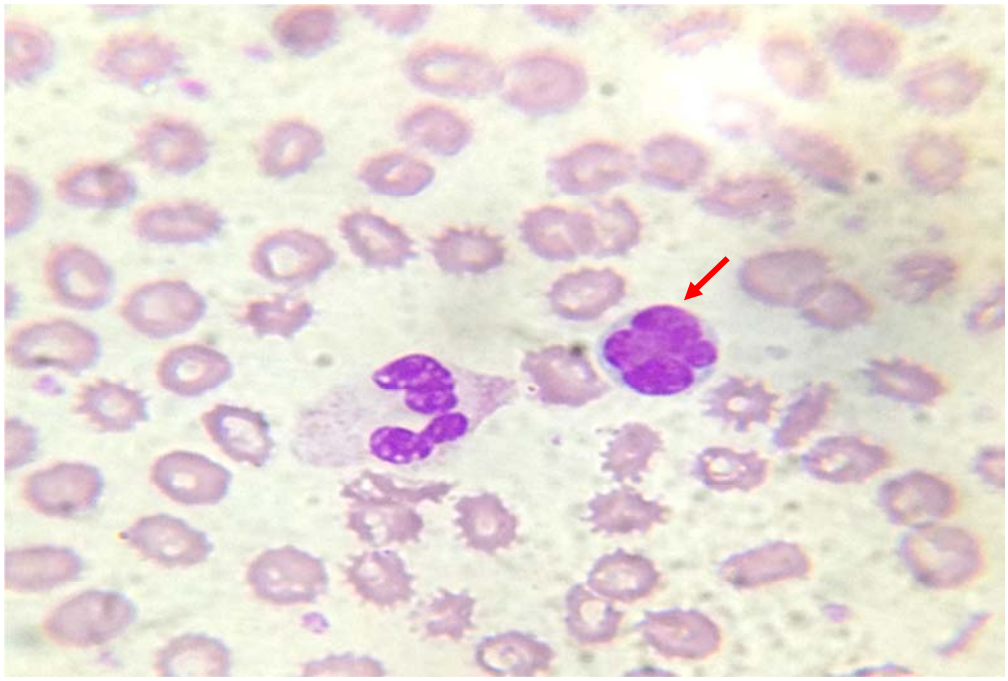


Colour Plate 4 : Peripheral smear showing small lymphocyte and a reactive lymphocyte (arrow) in Leishman stain (100x)

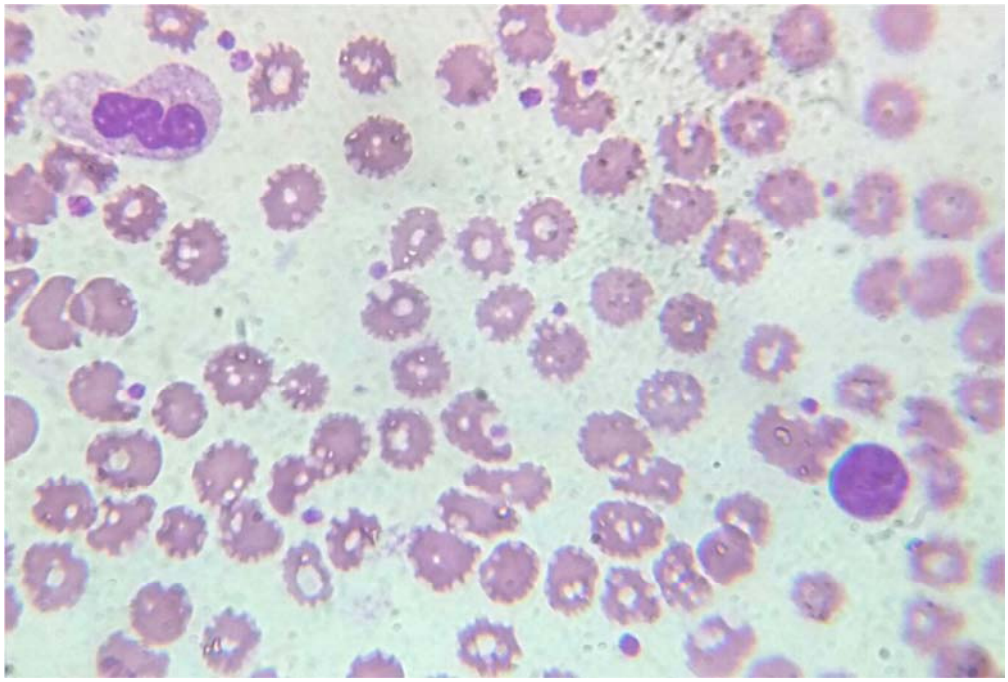
Colour Plate 5: Peripheral smear showing mature lymphocyte (arrow) with two eosinophils in Leishman stain (100x)



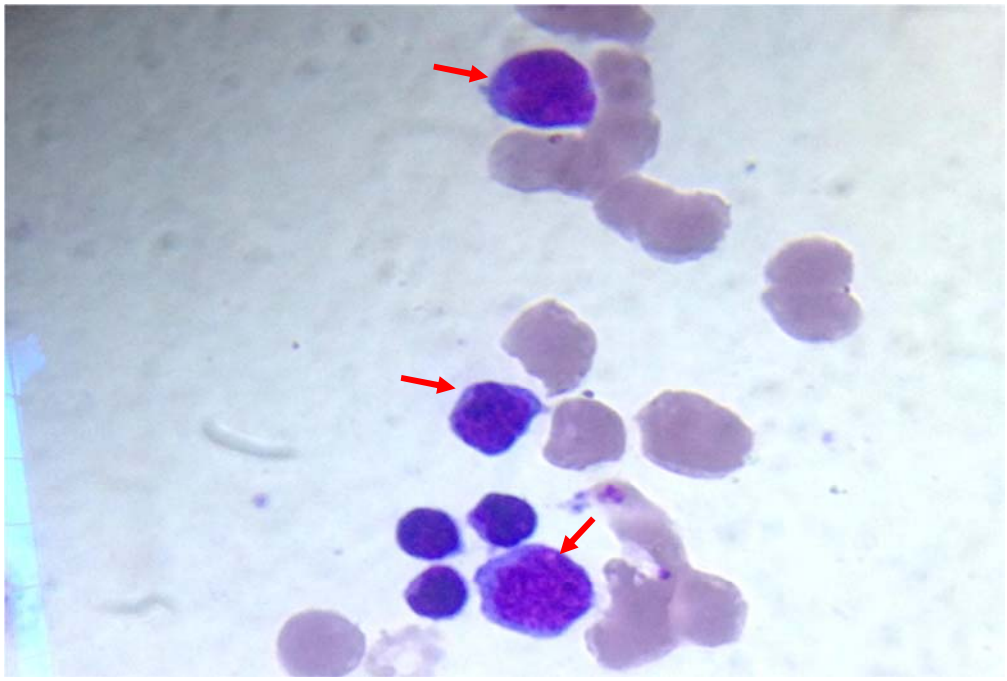
Colour Plate 6 : Peripheral smear showing band form of neutrophil with mature lymphocyte in Leishman stain (100x)



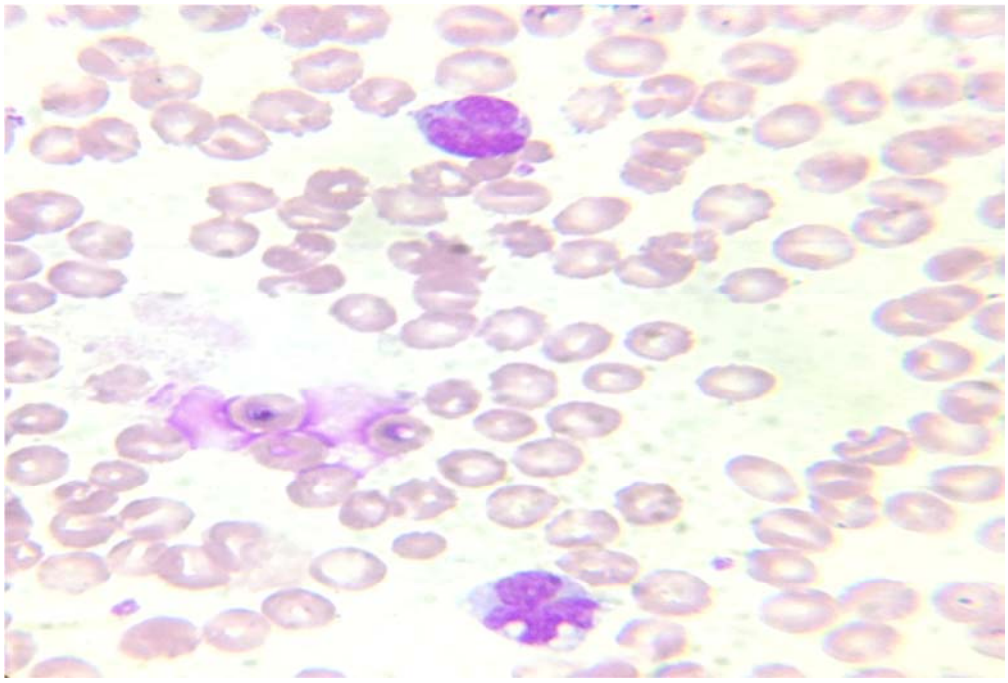
Colour Plate 7: Peripheral smear showing neutrophil with a reactive lymphocyte (arrow) in Leishman stain (100x)



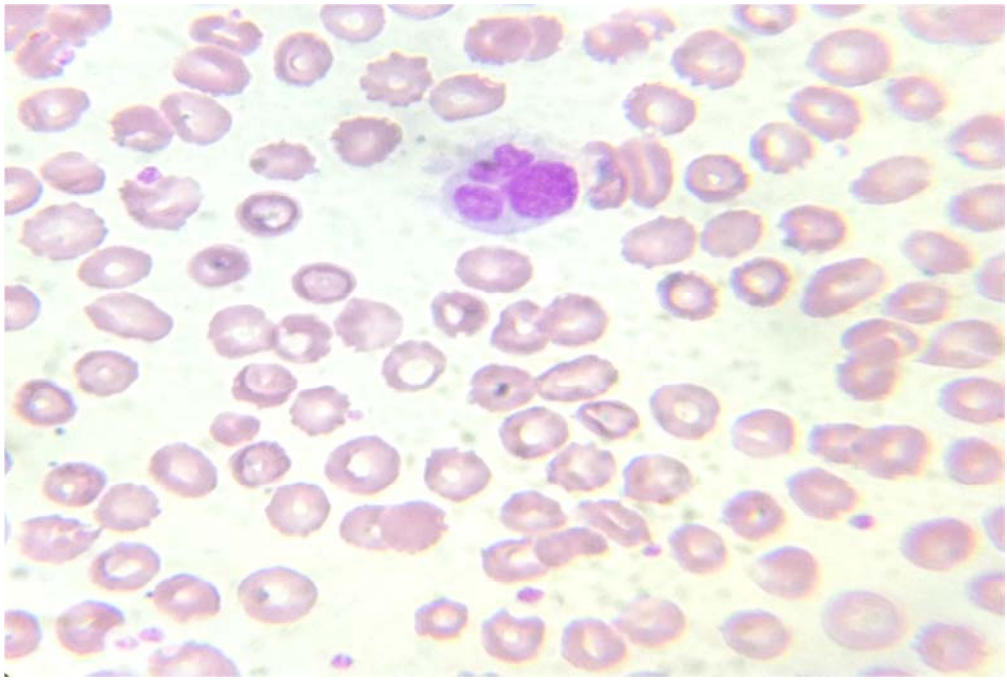
Colour Plate 8: Peripheral smear showing neutrophil with toxic change and a mature lymphocyte in Leishman stain (100x)



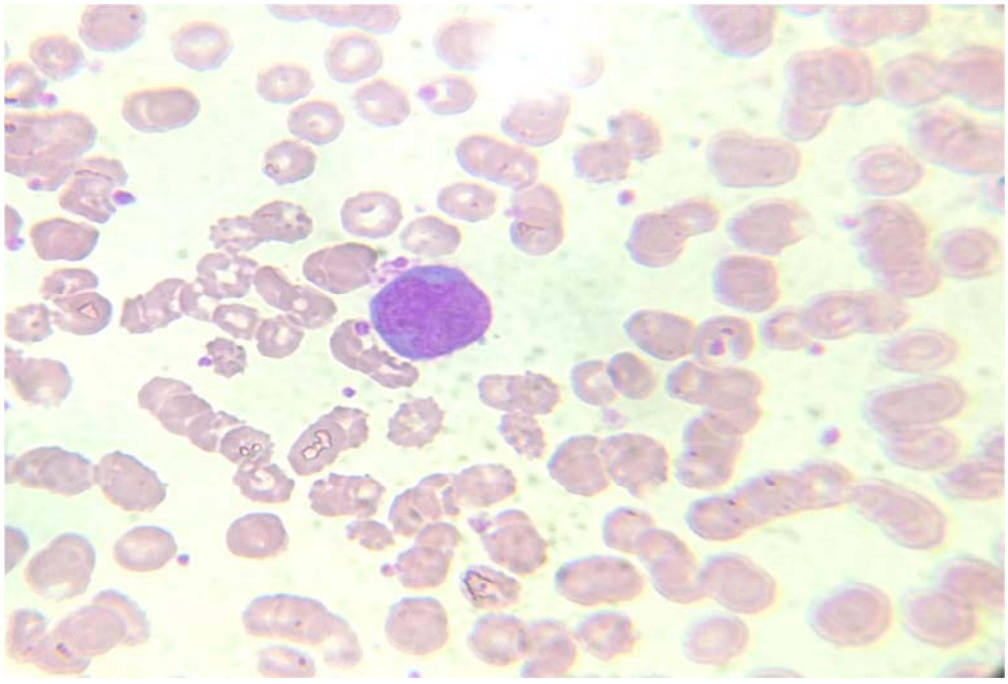
Colour Plate 9: Peripheral smear showing reactive lymphocytes (arrows) with normal mature lymphocytes in Leishman stain (100x)



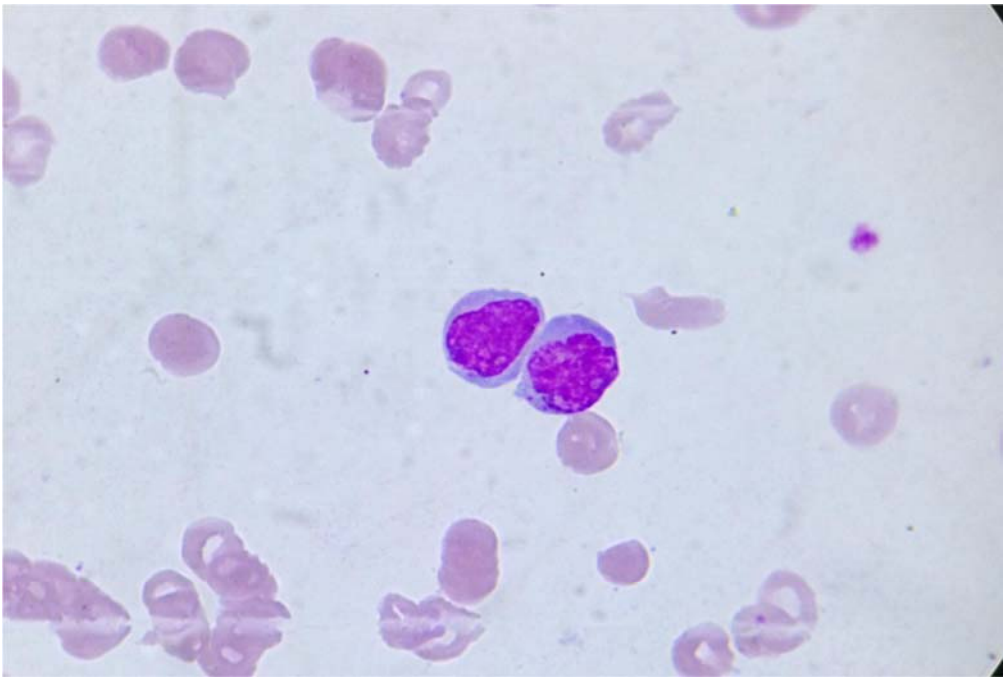
Colour Plate 10: Peripheral smear showing different morphologies of reactive lymphocyte in Leishman stain (100x)



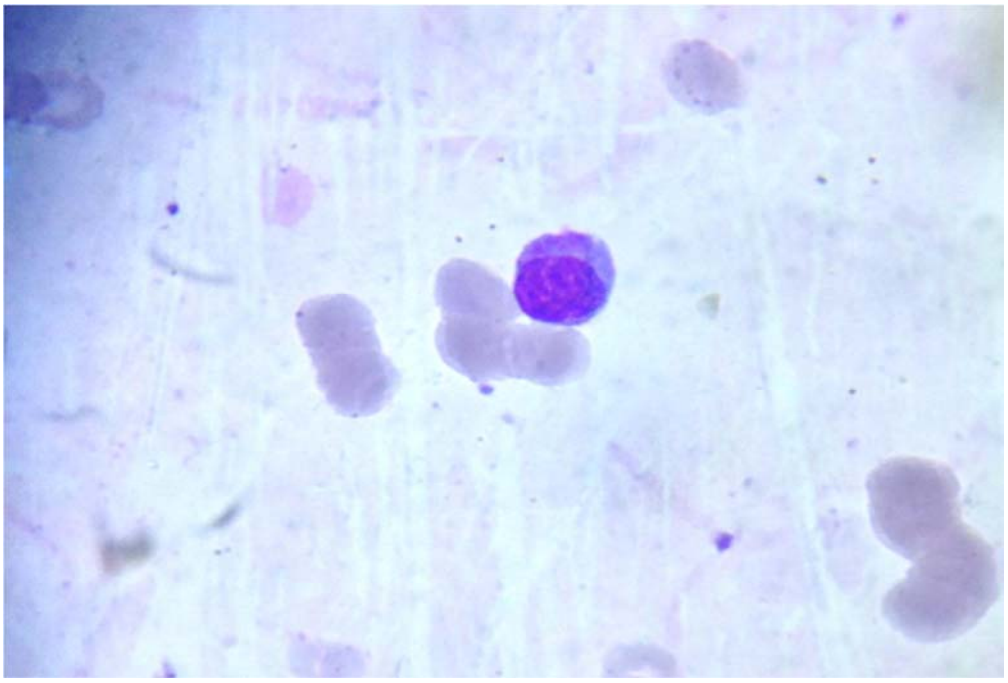
Colour Plate 11: Peripheral smear showing reactive lymphocyte with nuclear fragmentation in Leishman stain (100x)



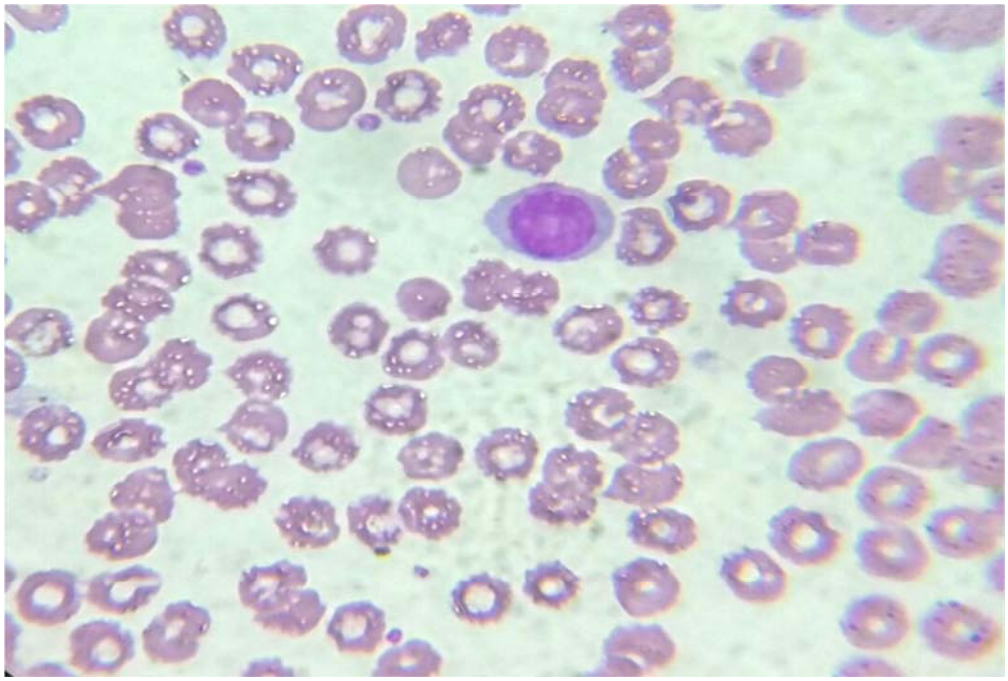
Colour Plate 12: Peripheral smear showing reactive lymphocyte - a different morphology in Leishman stain (100x)



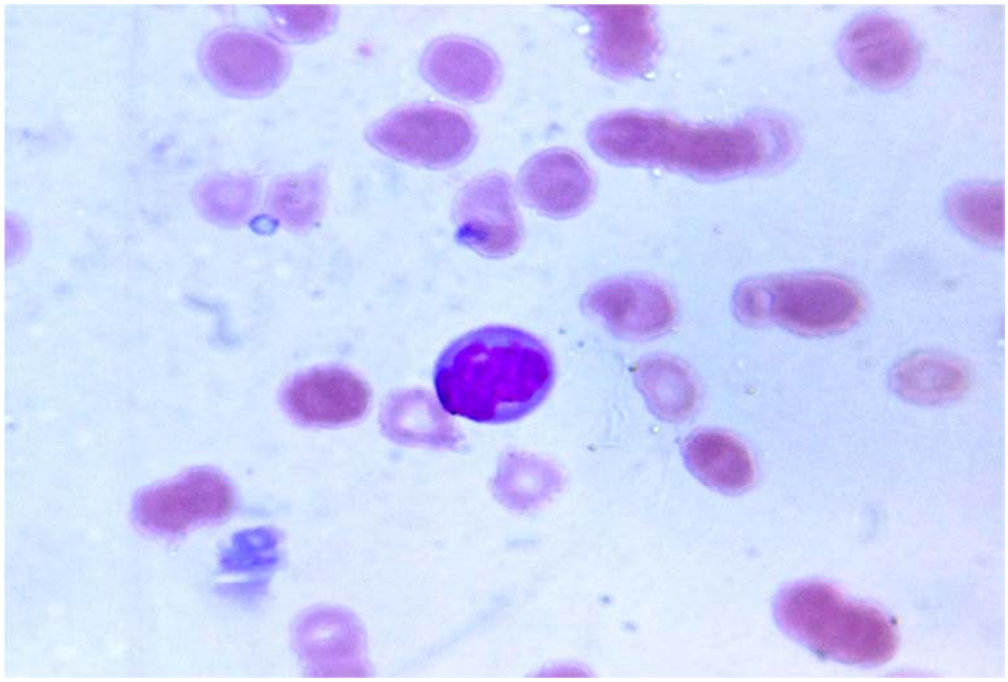
Colour Plate 13: Peripheral smear showing reactive lymphocytes with different morphologies in Leishman stain (100x)



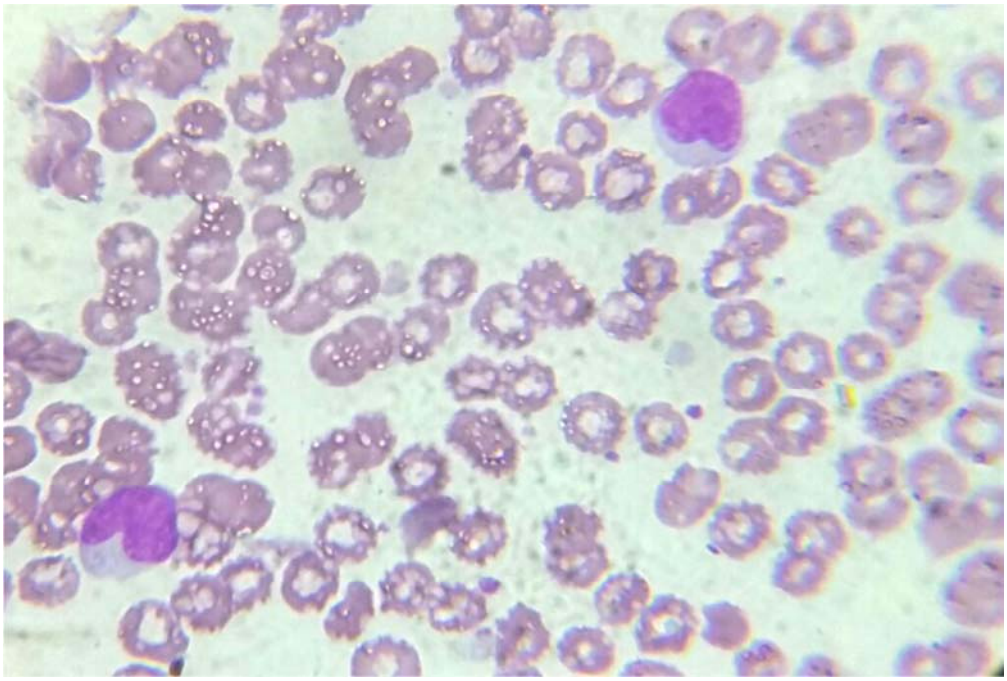
Colour Plate 14: Peripheral smear showing reactive lymphocyte in Leishman stain (100x)



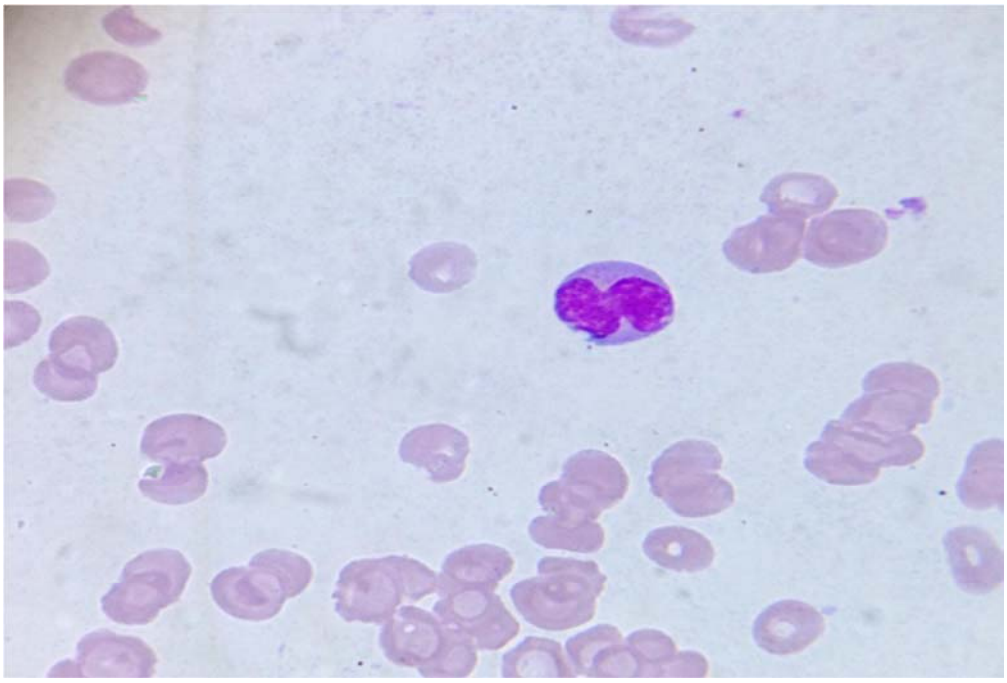
Colour Plate 15: Peripheral smear showing reactive lymphocyte in Leishman stain (100x)



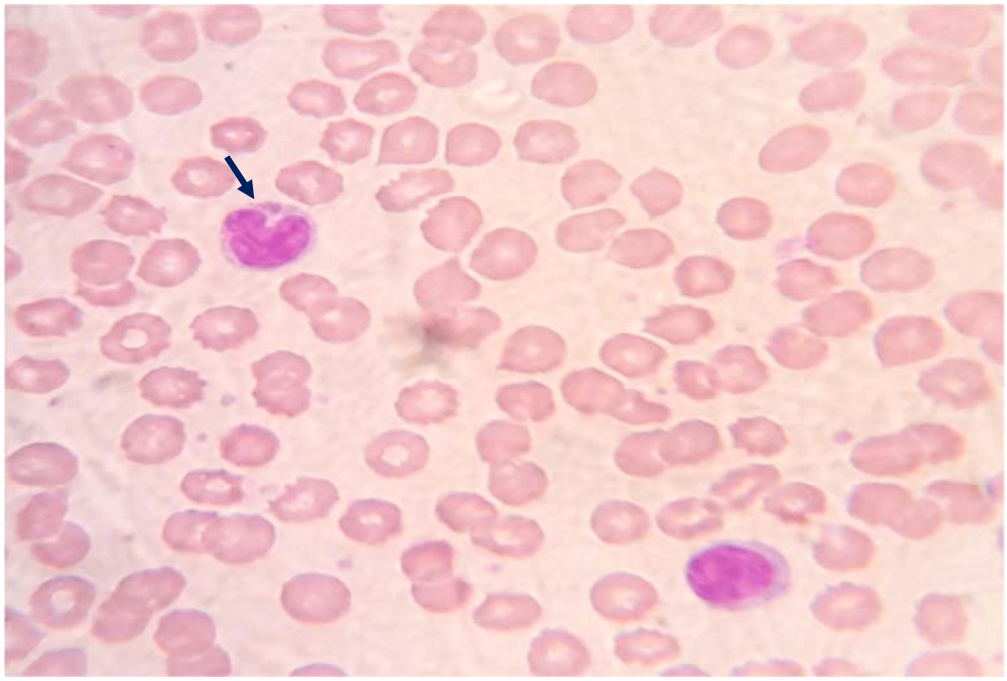
Colour Plate 16: Peripheral smear showing reactive lymphocyte with abundant cytoplasm in Leishman stain (100x)



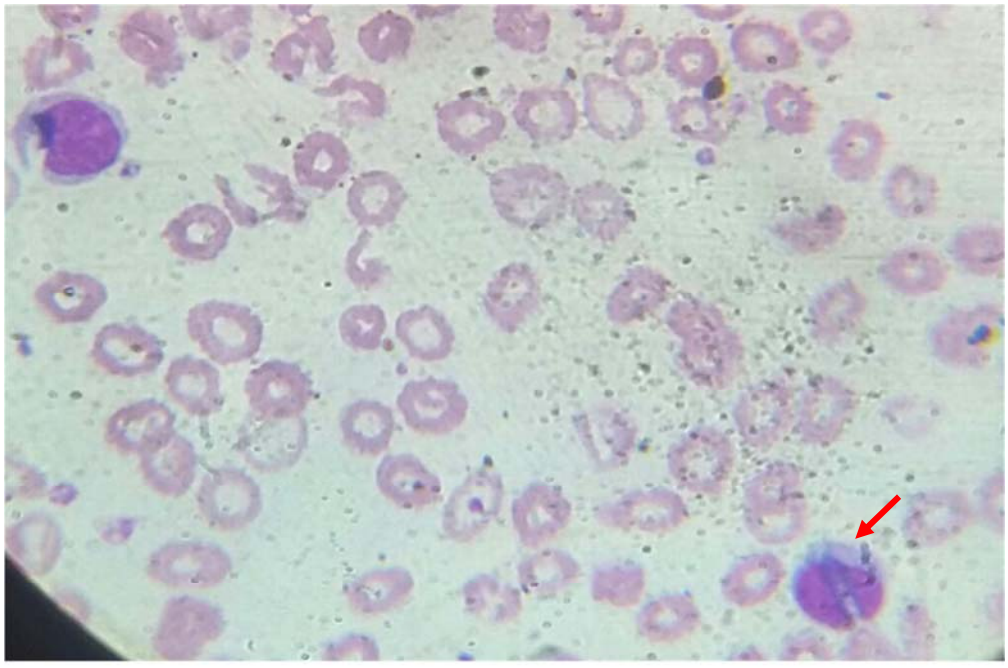
Colour Plate 17: Peripheral smear showing reactive lymphocyte with indendation in Leishman stain (100x)



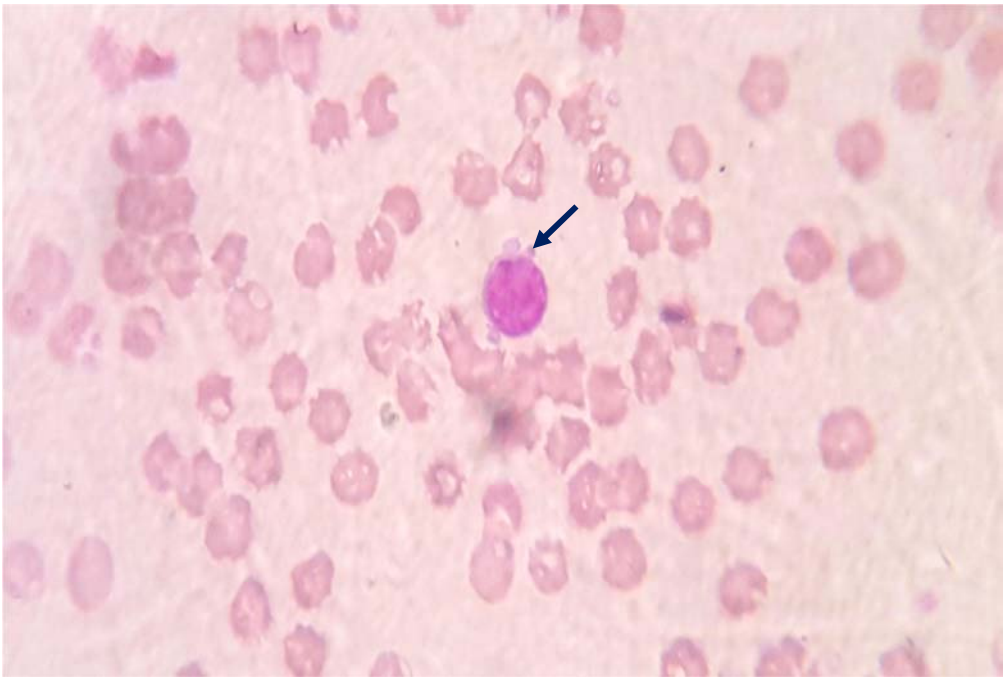
Colour Plate 18: Peripheral smear showing reactive lymphocyte with increased indendation of nucleus in Leishman stain (100x)



Colour Plate 19: Peripheral smear showing lymphocyte with increased indendation (arrow) similar to that of band form of neutrophil in Leishman stain (100x)

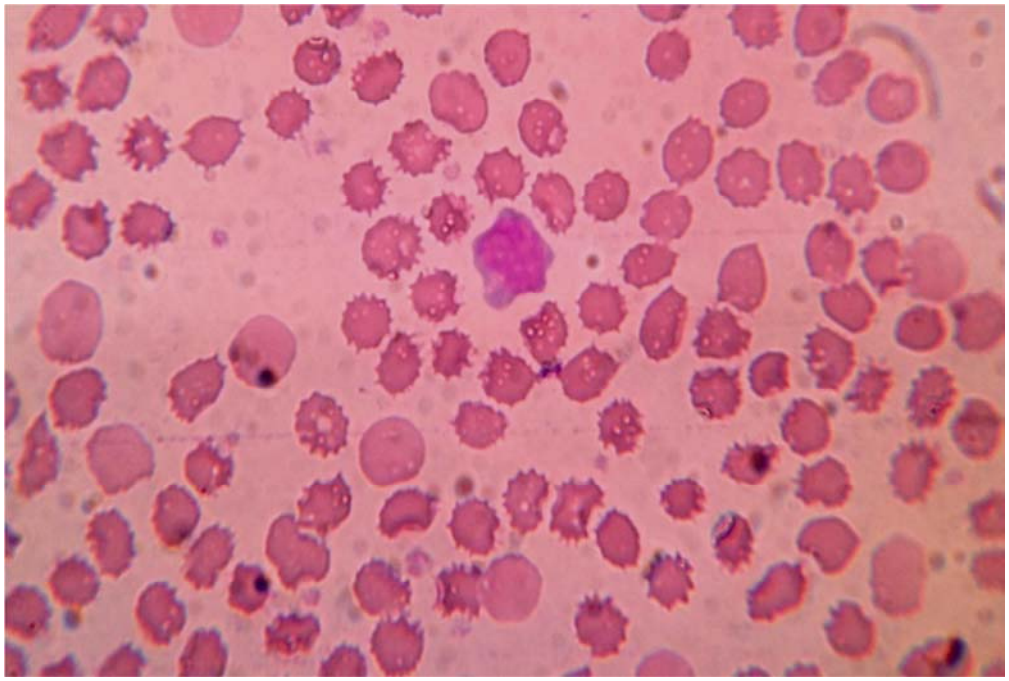


Colour Plate 20 : Peripheral smear showing reactive lymphocyte with increased nuclear indendation- binucleate form (arrow) in a smoker - Leishman stain (100x)

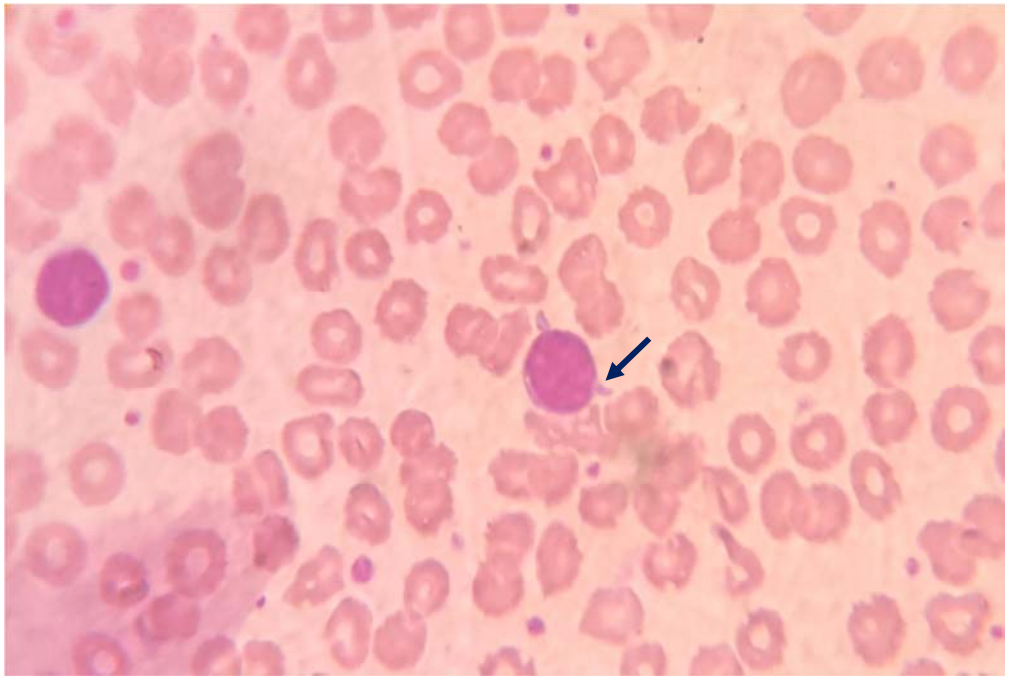


Colour Plate 21: Peripheral smear showing reactive lymphocyte with bleb formation in Leishman stain (100x)

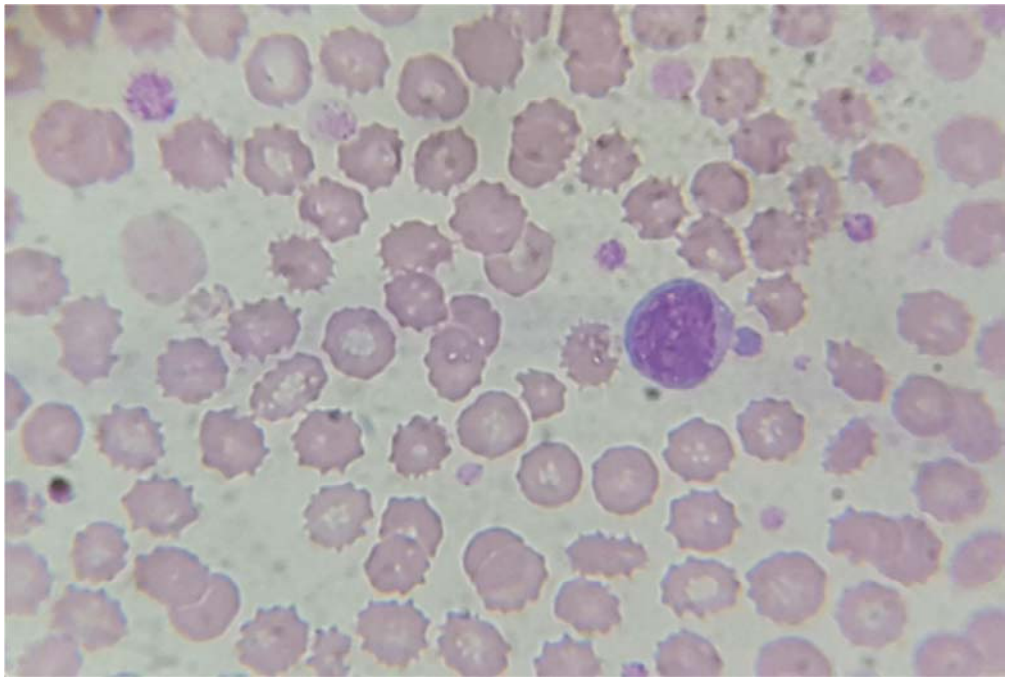
Colour Plate 22: Peripheral smear showing reactive lymphocyte with tiny cytoplasmic bleb in Leishman stain (100x)



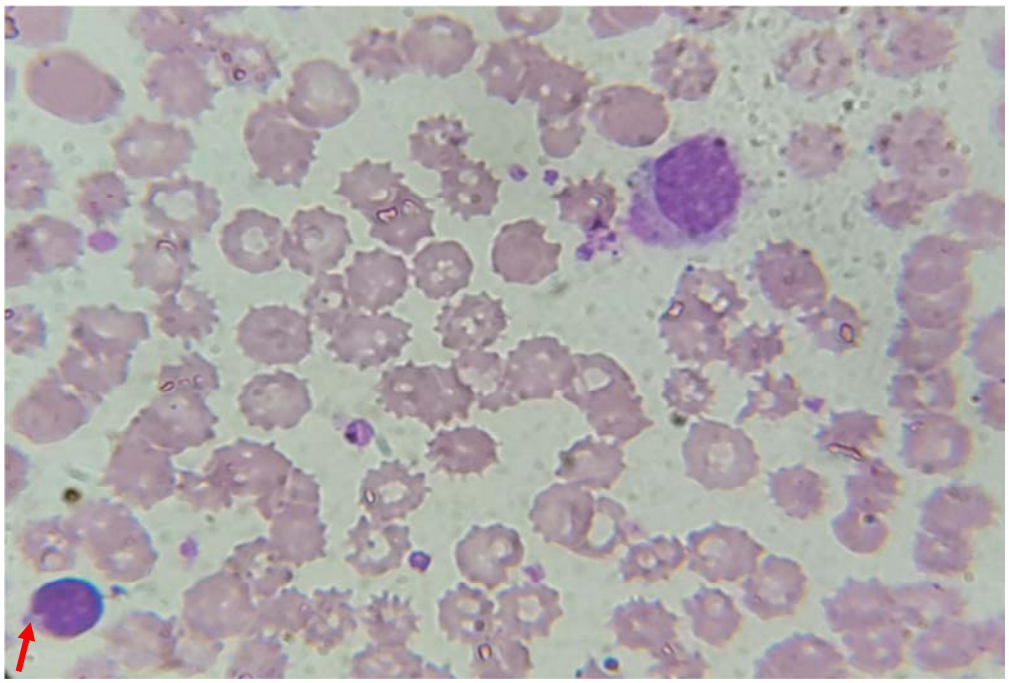
Colour Plate 23: Peripheral smear showing lymphocyte with blebs in the cytoplasm - Leishman stain (100x)



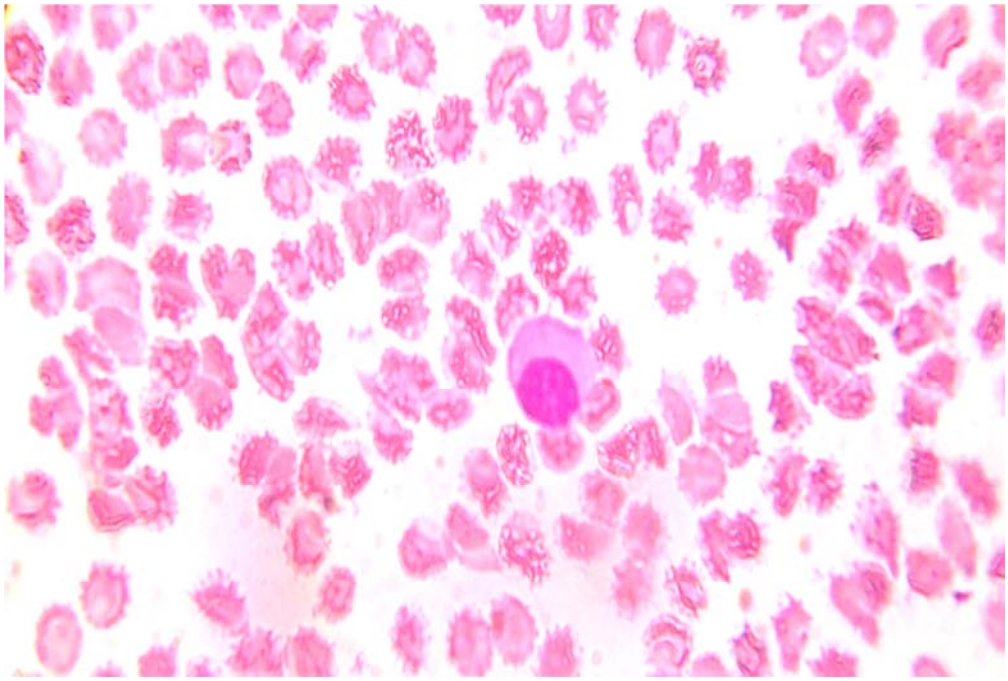
Colour Plate 24: Peripheral smear showing lymphocyte mimicking blast with blebs looking like horn (arrow) in Leishman stain (100x)



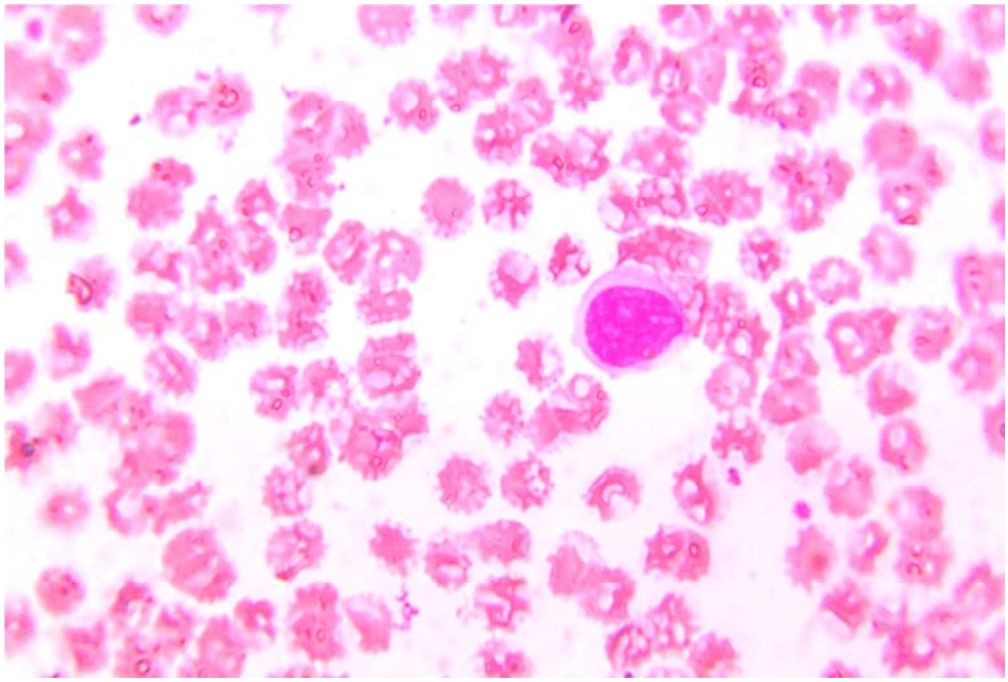
Colour Plate 25: Peripheral smear showing lymphocyte with knob like projection in the cytoplasm - Leishman stain (100x)



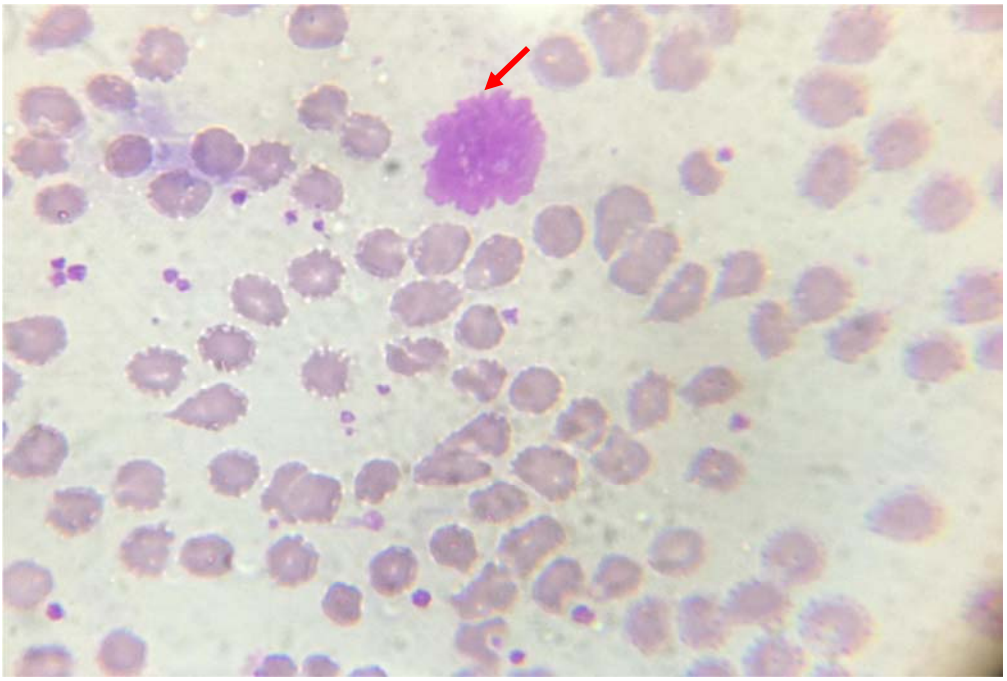
Colour Plate 26: Peripheral smear showing lymphocyte with knob like projection in the cytoplasm (arrow) and a reactive lymphocyte in Leishman stain (100x)



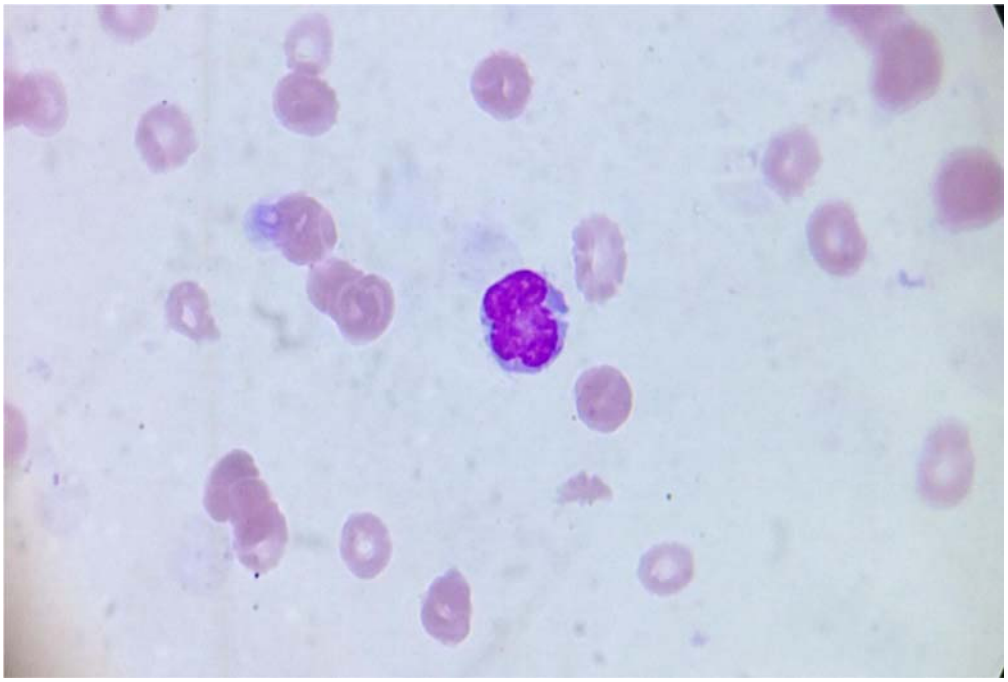
Colour Plate 27: Peripheral smear of dengue patient showing plasmacytoid lymphocyte in Leishman stain (100x)



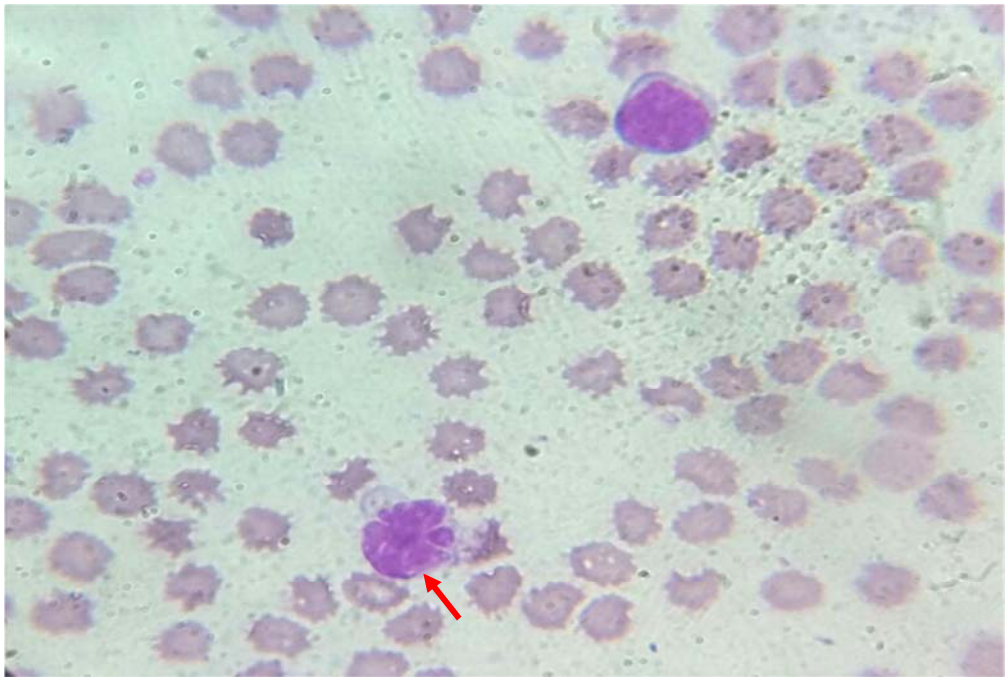
Colour Plate 28: Peripheral smear of dengue patient showing reactive lymphocyte with conspicuous nucleoli in Leishman stain (100x)



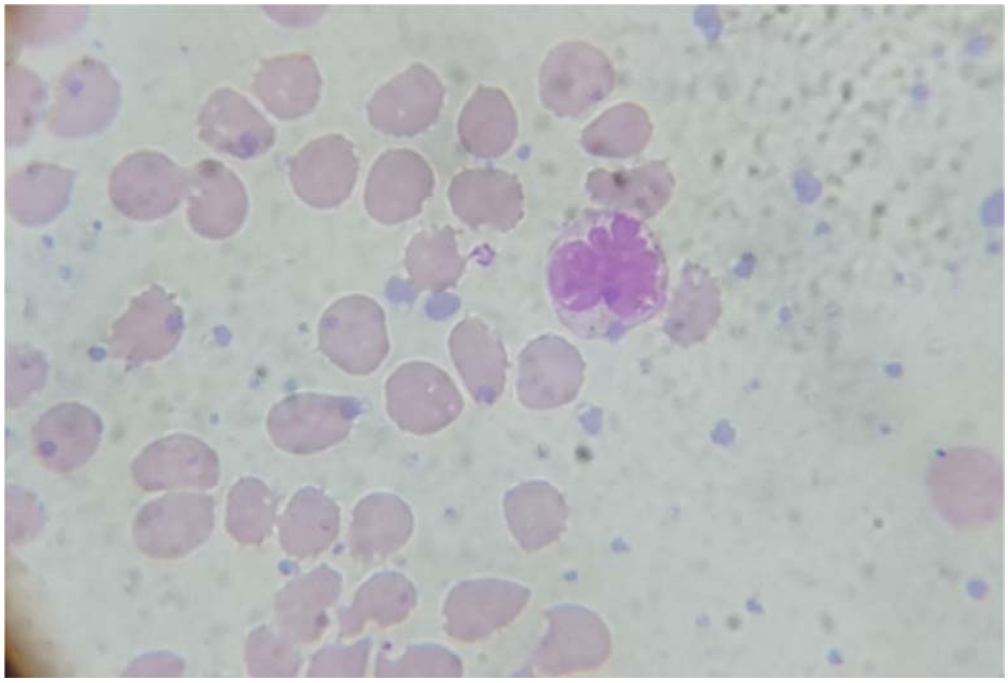
Colour Plate 29: Peripheral smear of a patient with fever showing smudge cell in Leishman stain (100x)



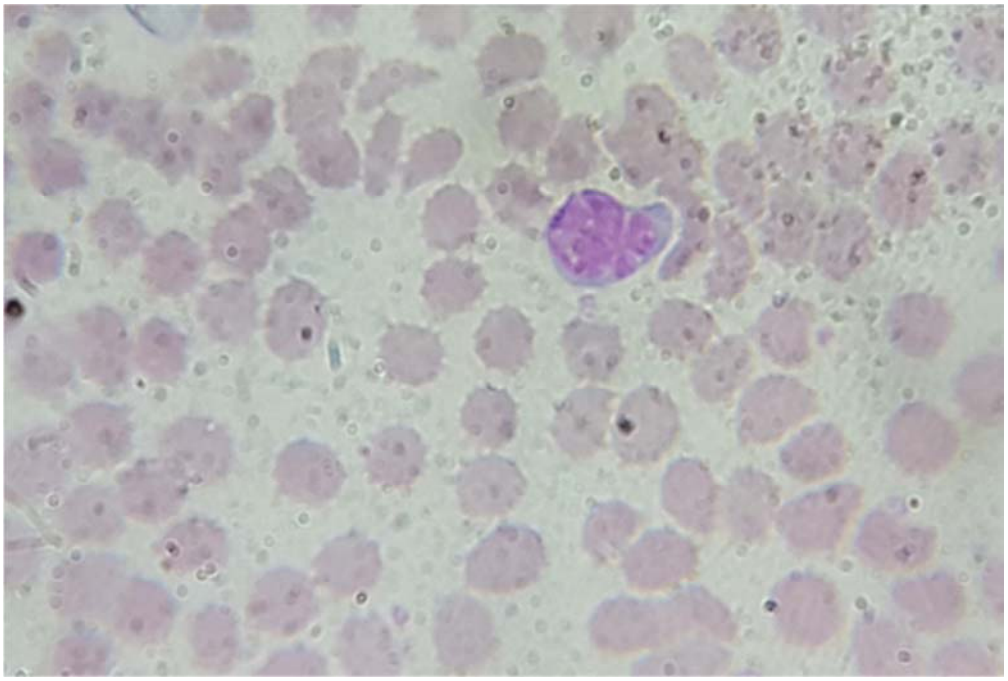
Colour Plate 30: Peripheral smear showing reactive lymphocyte - flower shaped nucleus in Leishman stain (100x)



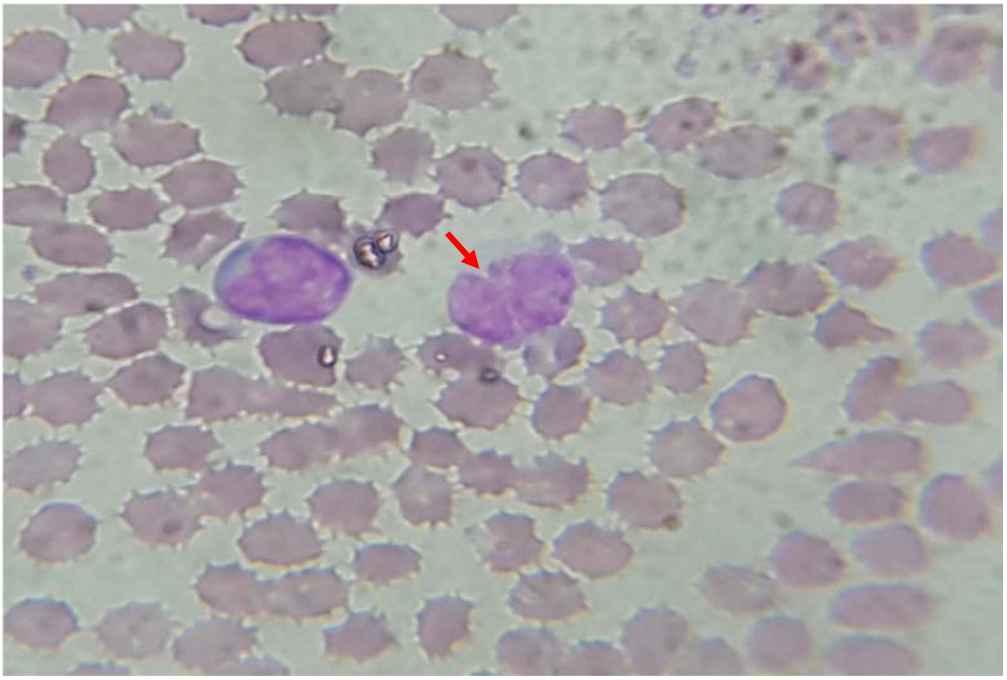
Colour Plate 31: Peripheral smear showing two reactive lymhphocytes, one of the lymphocyte showing flower shaped morphology (arrow) - Leishman stain (100x)



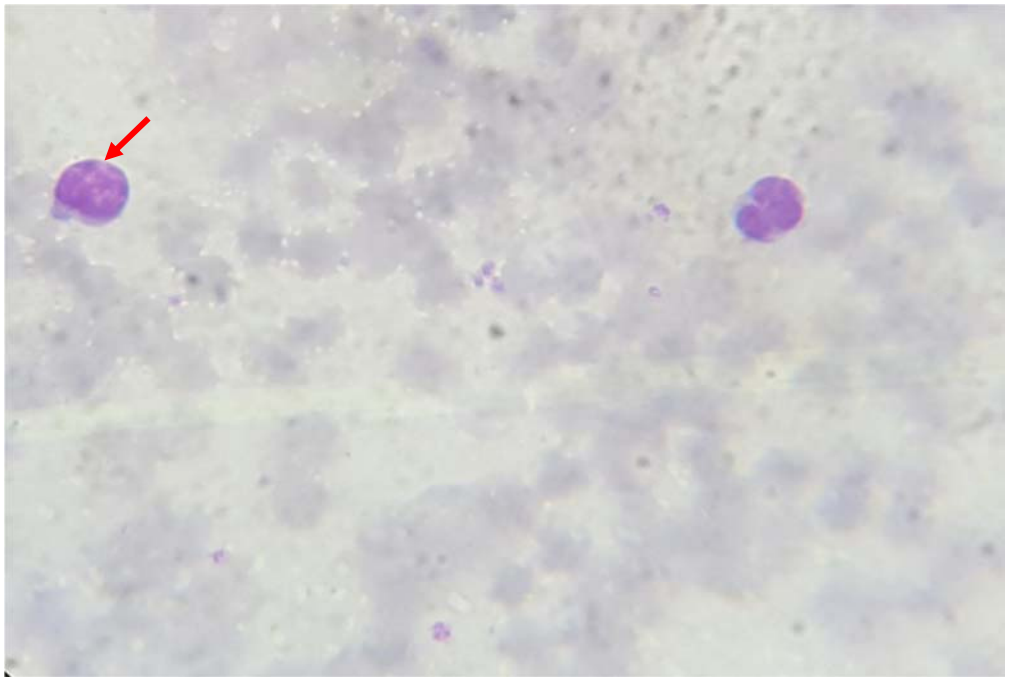
Colour Plate 32: Peripheral smear showing flower shaped reactive lymphocyte in Leishman stain (100x)



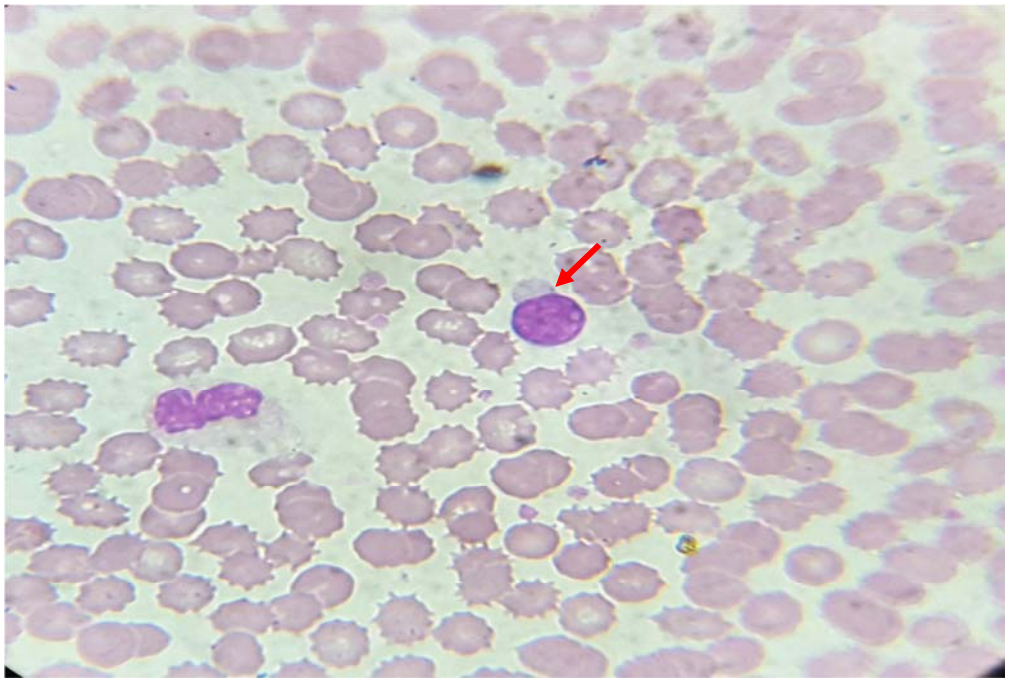
Colour Plate 33: Peripheral smear showing reactive lymphocyte with lobation and cleaving in Leishman stain (100x)



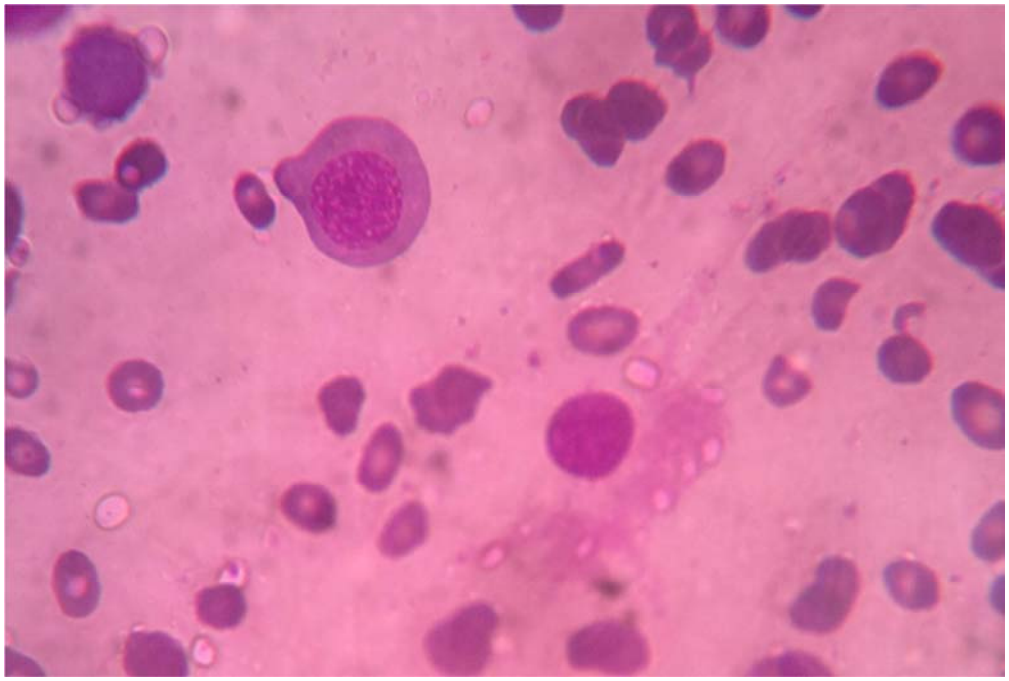
Colour Plate 34: Peripheral smear showing reactive lymphocyte with cleaving (arrow) along with a reactive lymphocyte in Leishman stain (100x)



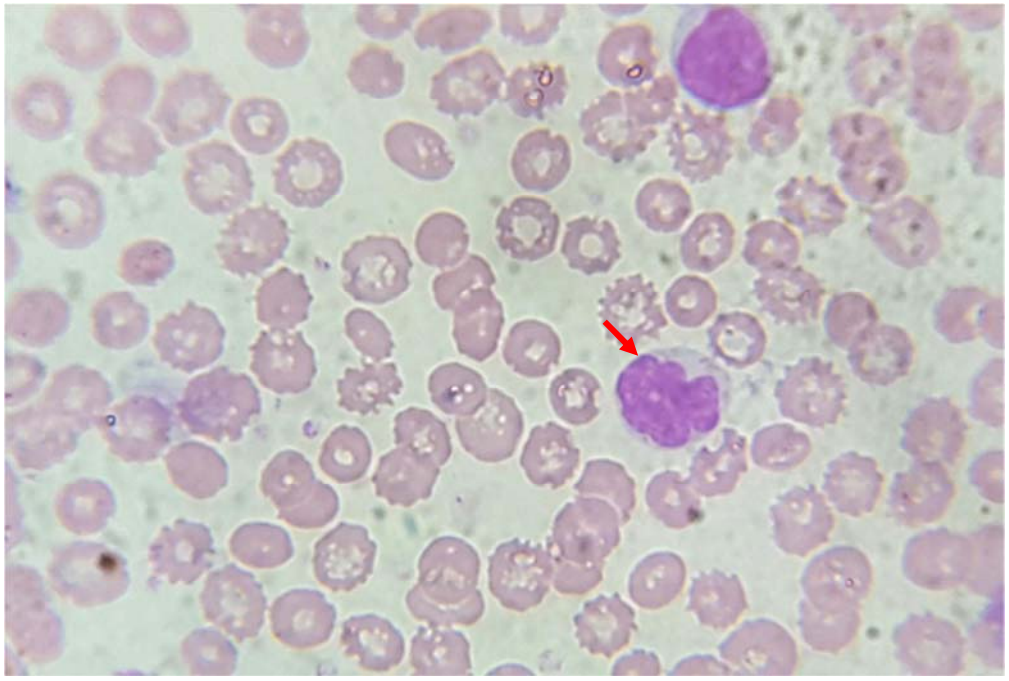
Colour Plate 35: Peripheral smear showing reactive lymphocyte which is opened up and slightly larger (arrow) than the other lymphocyte in Leishman stain (100x)



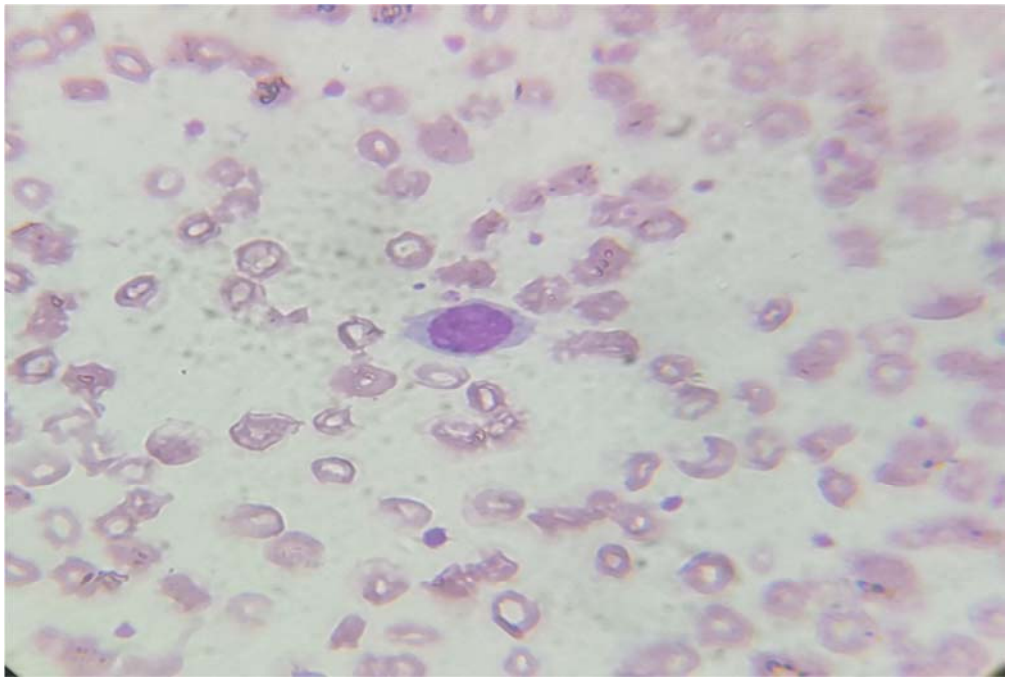
Colour Plate 36 : Peripheral smear showing hand mirror like (arrow) lymphocyte in Leishman stain (100x)



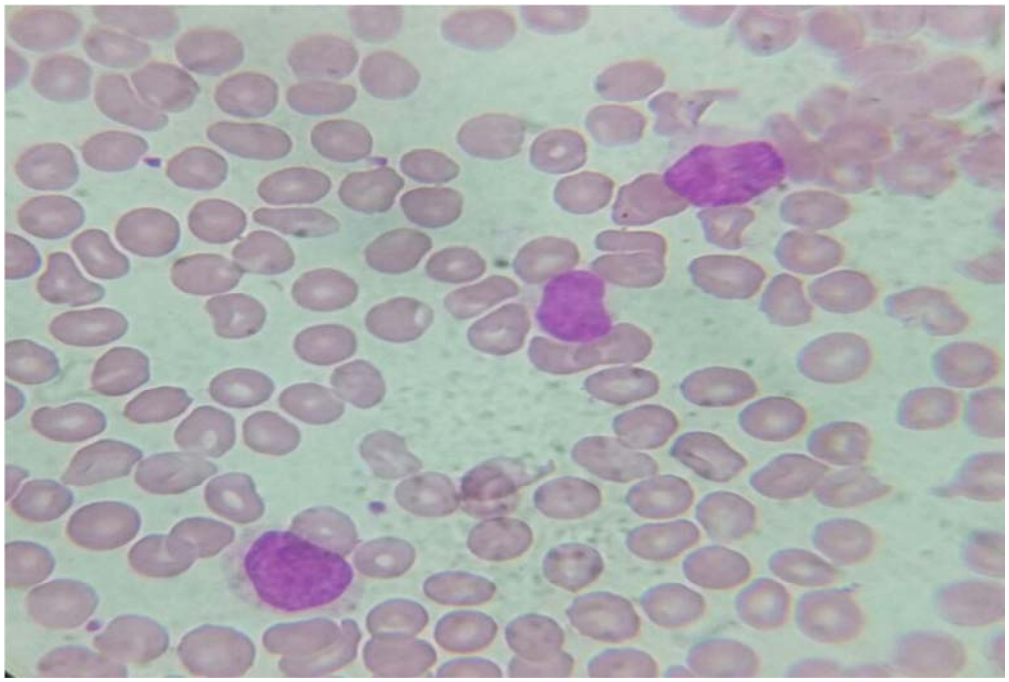
Colour Plate 37 : Peripheral smear showing reactive lymphocyte with hand mirror appearance in Leishman stain (100x)



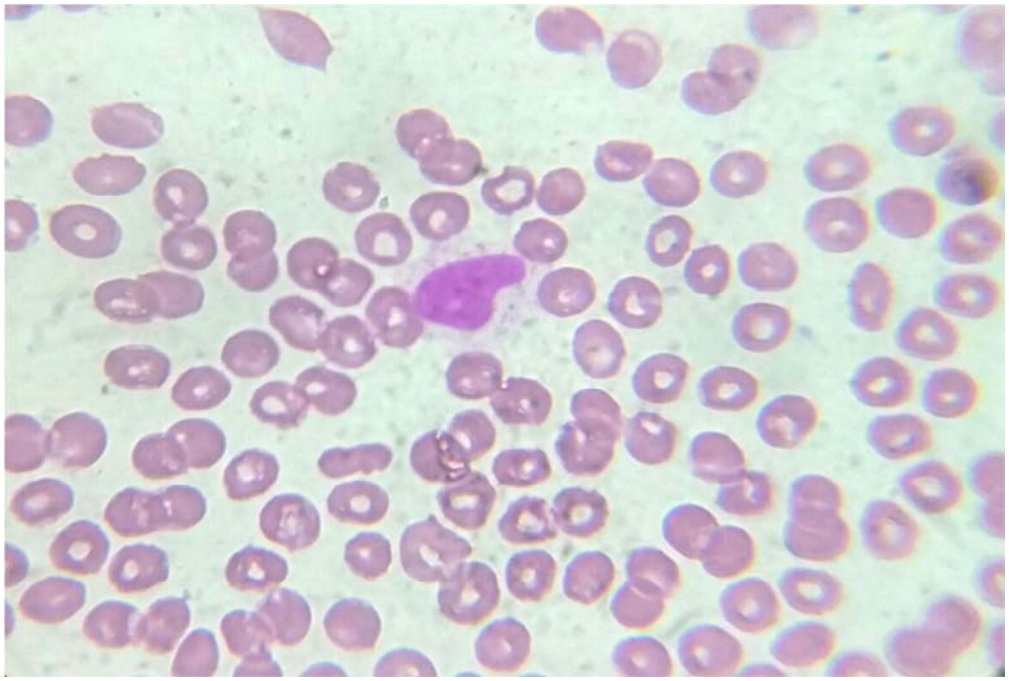
Colour Plate 38 : Peripheral smear showing folded nucleus of lymphocyte with nucleoli (arrow) in Leishman stain (100x)



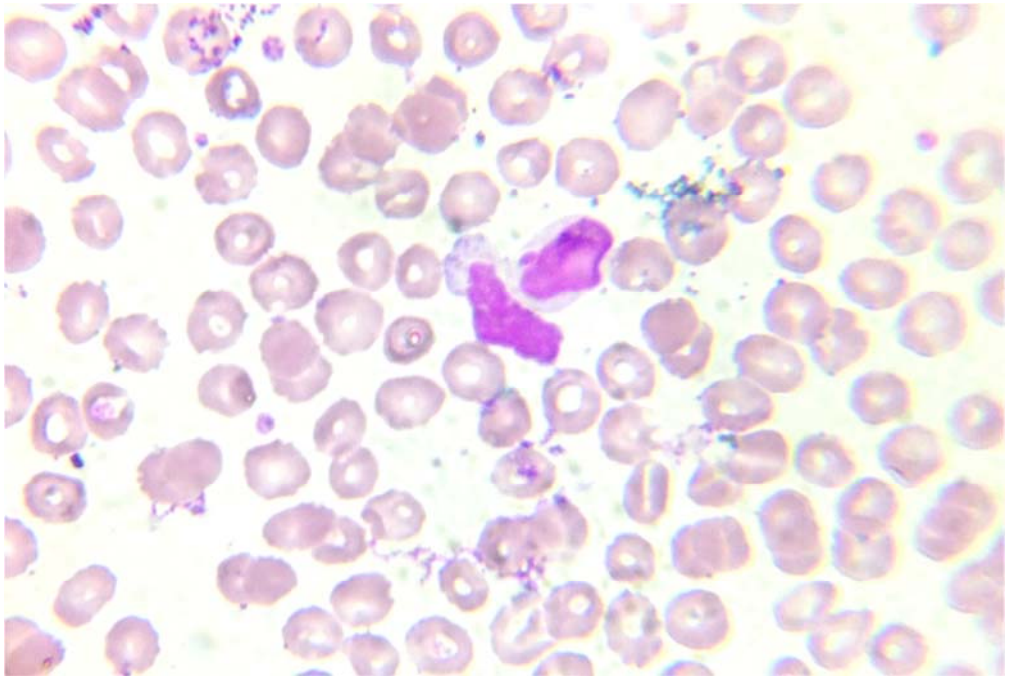
Colour Plate 39: Peripheral smear showing lymphocyte with abundant bipolar cytoplasm mimicking malignant lymphocytes in splenic mantle cell lymphoma in Leishman stain (100x)



Colour Plate 40: Peripheral smear showing atypical lymphocytes in patient with upper respiratory tract infection - Leishman stain (100x)



Colour Plate 41 : Peripheral smear showing atypical lymphocyte in a dengue patient - Leishman stain (100x)



Colour Plate 42 : Peripheral smear showing atypical lymphocyte in a patient with malaria - Leishman stain (100x)

Discussion

DISCUSSION

In this era, with the available sophisticated methods used as a diagnostic tool, still the peripheral smear examination plays a major role in diagnosis of various diseases. The various reactive forms of lymphocytes studied under light microscopy gives us a clue of a reactive process which can sometimes be misleading to a differential diagnosis of a neoplastic condition.

Lymphocytosis is seen commonly in patients with viral etiology, tuberculosis and lymphoproliferative diseases and a cut off range for absolute lymphocytosis is not clearly defined in literature. The need for morphological evaluation of lymphocytes in lymphocytosis is essential to prove a reactive or a malignant course of disease. This study evaluates the morphology of lymphocytes in patients with an absolute lymphocyte count more than 3000/ul and patients with an absolute lymphocyte count more than 4000/ul to select the cut off range for further evaluation. The different morphologies of a lymphocyte which mimics various conditions described in literature, were also studied.

Relation to stress:

In the study by Nitin.J.Karandikar et al in 52 patients whose initial absolute lymphocyte count was more than 4000/ul initilally following an

acute stressful event along with presence of various morphological forms of reactive lymphocytes[8], which were termed as transient stress lymphocytosis, showed reversal to normal lymphocyte count and morphology within 24 to 48 hours post the stressful event. In this study, 23 patients reported initially with an acute stressful event, majority of them had an absolute lymphocyte count more than 4000 with reactive lymphocytes in the peripheral smear. On follow up after a period of three to six months, the lymphocytes from these patients showed a normal morphology and the absolute lymphocyte count had also reduced. (Table 3, Table 7, Chart16)

Relation to respiratory infection:

There were few atypical lymphocytes viewed in peripheral smear in the patients with complaints of fever and upper respiratory tract infection in this study, which showed a reversal of morphology after a few weeks time and appropriate clinical treatment.(Colour Plates 27,28,29,40,41) Similarly, in literature, Suresh Venugopal et al. had reported a case of a atypical lymphocyte in upper respiratory tract infection associated with streptococcal infection[20].

Atypical lymphocytes were noted in the peripheral smear of a patient with malaria in this study (Colour Plate 42, Chart 11). In literature, Burke A Cunha et al has studied and proven the association of atypical lymphocytes in acute malarial infection.[72]

Relation to skin and psychiatric ailments:

In this study, 7 cases had come with skin related complaints like utricaria, psoriasis or hypersentivity reactions and 7 cases with psychiatric episodes. Most of these patients showed an increase in absolute lymphocyte count more than 4000 and also showed reactive lymphocytes in their peripheral blood. On reviewing these patients blood smears after a period of 3 to 6 months, all of them had a reversal of morphology of the lymphocytes to a mature form, with reduction in absolute lymphocyte count as well. (Chart 16)

Relation to smoking:

In this study, 31 patients had a history of smoking. More than 50% of these patients showed an absolute lymphocyte count of more than 4000 and about 60% of the 31 cases showed a reactive lymphocyte morphology(Chart17,18,19). In literature, Dasanu et al[61] has studied a population of 21 patients with chronic smoking history and all of them showed B cell lymphocytosis with reactive form of lymphocytes[61]. Xavier Troussard et al had quoted that a majority population of absolute lymphocytosis associated with chronic smoking shows binucleate reactive forms of lymphocytes[22]. In this study, lymphocytes with binucleate forms were seen in smokers (Colour Plate 20), which reverted to normal morphology after abstinence from tobacco and smoking for 3 to 6 months.

In patients with alcoholism, there was no distinct association with lymphocyte count or morphology noted after cessation of the habit.

In this study, of the 120 patients assessed, 80 patients had an absolute lymphocyte count more than 4000/ul and 40 had an absolute lymphocyte count between 3000 to 4000/ul. Of the 80 patients with ALC more than 4000/ul, 68 patients showed a reactive morphology of lymphocyte. Whereas, in the 40 patients with an ALC between 3000/ul to 4000/ul, 17 of them showed reactive forms of lymphocytes. This gives the information that it is essential to carefully examine all the peripheral smears with an ALC above 4000 as they mostly show a morphological variation in lymphocyte which can give us a clue to diagnosis.

In the study by Sun P et al, 2014, 71 adult patients with an absolute lymphocyte count more than 5000/ul were studied and 80% of the study population showed reactive lymphocytosis[26]; the peripheral smear of these subjects on review revealed a reactive process. However, in the study by Tseng V et al[26], 2014 , in a study population of 1170, morphological evaluation of lymphocytes above an ALC of 5000/ul only was proved to be essential to validate a reactive process by peripheral smear study.

This study closely resembles the study by Andrews JM et al[37] with a cut off value of >4000/ul of ALC for the morphological evaluation of lymphocytes.

TABLE 9 : Comparison of ALC Cut Off Range for Morphological Evaluation With Similar Studies

STUDY	ALC Cut Off Value for Morphological Evaluation of Lymphocyte
Present study	**>4000/ul**
Tseng V et al[26]	>5000/ul
Sun P et al[36]	>7000/ul
Andrews JM et al[37]	>4000/ul

The various morphologies demonstrated in our study include lymphocytes with cytoplasmic blebs, reactive lymhpocytes with hand mirror appearance and also lymphocytes with bipolar cytoplasm.

Cytoplasmic blebs in hematology are typical morphological appearance seen in a megakaryoblast[56](Colour plates 21-24) In literature, Tirado et al has proven that lymphocytes with blebs can be seen in cases of T cell prolymphocytic leukemia[57.] Sanderson et al has proven that lymphocytes have cytoplasmic blebbing when The T cells are needed for target cell killing as a mechanism of cytotoxicity[58]. In this study, many reactive lymphocytes have shown to morphologically appear with cytoplasmic blebbing. These patients with such morphological type of lymphocytes have normal mature type of lymphocytes in their review peripheral smears after a period of 3- 6 months. This gives us an insight

that cytoplasmic blebbing in lymphocytes can be due to a reactive processes.

Lymphocytes with hand mirror appearance denote a neoplastic process. In literature, WJ Tomas et al's study in 25 Infectious Mononucleosis patients has shown that 10 of them showed hand mirror like lymphocytes as a reactive process in relation to cytotoxicity[59]. In our study, two patients with fever have shown to have hand mirror like appearance of lymphocytes which were not to be seen after treatment and a follow up a period of 3 months suggesting that such morphological variations in lymphocytes are due to the cytotoxicity of lymphocytes which are seen in a reactive process. (Colour plates 36,37)

In literature, Tracy I George et al, 2012 has mentioned that bipolar cytoplasmic projections, known as villous lymphocytes[69] are seen in splenic mantle cell lymphomas[60]. In this study, two cases with complaints of fever showed cytoplasmic projections in the lymphocytes suggesting a bipolar cytoplasm. Further investigations on the patients were inconclusive and after a course of antibiotics and smoking abstinence, both the patients showed mature lymphocytes on follow up. (Color Plate 39)

Normal lymphocyte count by the cell counter method is 20-40%[4]. This study has given us information that reactive lymphocyte morphology are found majorly in a lymphocyte count more than 35%. There is

predominance of mature type of lymphocytes when the lymphocyte count is between 20 -35%. This shows that there may be need of evaluation of lymphocyte morphology when the cell counter gives a lymphocyte value more than 35 %, depending upon the clinical parameters. (Chart 15)

In literature, absolute lymphocytosis along with reactive lymphocytes are studied in patients with thrombocytopenia and patients presenting with neutropenia[62]. In this study, there was no significant correlation between the neutrophil count with the lymphocyte count or morphology. Similarly no significant correlation was sorted out with the platelet count in relation to the count and morphology of the lymphocytes. (Chart 8, Chart 9)

In this study, biochemical parameters included were LDH and C-reactive protein. For the total 9 patients subjected to these studies, only one patient with elevated LDH count showed reactive lymphocytosis. Thereby, correlation between the biochemical parameters, lymphocyte count and its morphology were not conclusive.

Summary

SUMMARY

Absolute lymphocyte count gives us a information whether further evaluation of the morphology of lymphocytes is essential or not. This study has shown that the majority of patients with an absolute lymphocyte count of more than 4000/ul show a variation in morphology of lymphocytes. Hence, a peripheral smear study of all cases with an absolute lymphocyte count more than 4000/ul is needed to distinguish them as a reactive process or a neoplastic process. All the cases with a reactive morphology and an absolute lymphocyte count more than 4000/ul have to be reviewed after a course of time to rule out persistence of reactive lymphocytes which can be due to an underlying neoplastic process.

Reactive lymphocytes can present with a varied number of morphologies which can sometimes mimic neoplastic type of lymphocytes and therby needs to be carefully examined to avoid false positive diagnosis. This study has shown that hand mirror appearance, bipolar cytoplasmic extensions in lymphocytes and cytoplasmic blebbing can be due to a reactive process as a mechanism of cytotoxity and not neccessarily suggest an underlying neoplastic process.

Reactive lymphocytes have many etiologies but their close association with acute stress induced history, chronic smoking, psychiatric illness and skin related problems seen in this study show that the reactive

change in lymphocytes does not pertain to viral infections or neoplastic process alone.

This study has mainly given us information on the cut off value for absolute lymphocytosis, morphology of lymphocytes and its clinical correlation. This study paves a platform for further studies on lymphocytes and its correlation with various hematological and biochemical parameters.

Conclusion

CONCLUSION

1. From the current study, it is inferred that an absolute lymphocyte count more than 4000/ul in adults needs a morphological evaluation of lymphocytes to evaluate the cause.

2. Varied morphologies of reactive lymphocytes can be seen in peripheral smears of absolute lymphocytosis which includes cytoplasmic blebbing, bipolar cytoplasm and hand mirror appearance which can mimic neoplastic type of lymphocytes and these cases are not to be misdiagnosed without complete clinical workup.

3. Absolute lymphocytosis with reactive forms of lymphocytes are clinically correlated to acute stress, smoking, respiratory infections, skin and psychiatric ailments.

4. Hematological parameters including platelet count, neutrophil count and biochemical parameters have not shown a significant correlation with the morphology of lymphocytes in patients presenting with absolute lymphocytosis.

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Annexures

PROFORMA

Name: H.NO: Address & ph no:

Age: Ward & Unit:

COMPLAINTS:

Past History:

Diabetes mellitus Yes ☐ No ☐

Previous Illness ☐ ☐

Personal History

Smoking ☐ ☐

Alcohol ☐ ☐

Examination

General Examination

Pallor ☐ Icterus ☐ Clubbing ☐ Cyanosis ☐ Lymphadenopathy ☐

Edema ☐

Lymphnode Examination :

Skin :

P/A examination : Hepatomegaly ☐ Splenomegaly ☐

INVESTIGATIONS:

CBC :

Haemoglobin	
PCV	
HCT	
Total Count	
Differential Count	Neutrophils : Lymphocytes : Eosinophils : Monocytes : Basophils :
Platelets	

Peripheral Smear Study:

RBC:

WBC:

Platelets:

Impression:

MP/MF : YES ☐ NO ☐

VIRAL SEROLOGY:

Comments:

Review :

INFORMED CONSENT

Undertaking by the investigator:

Your consent to participate in the research project titled "MORPHOLOGICAL EVALUATION OF LYMPHOCYTES ON PERIPHERAL SMEAR EXAMINATION IN ADULT PATIENTS WITH LYMPHOCYTOSIS AND ITS CLINICAL CORRELATION" is sought. You have the right to refuse consent or withdraw the same at any stage after being included. In such an event, you will receive the best possible treatment without any prejudice. If you have any doubts about the study you may discuss your concerns with the principal investigator.

CONSENT:

I ……. have been informed about the details of the research project. I have been explained the study in my own language and been given opportunity to ask questions and clarify my doubts. I understand that as a part of this study I have to undergo clinical examination and some investigations. I have understood that I have the right to refuse my consent or withdraw it anytime during the study without adversely affecting my treatment. I agree not to restrict the use of any data or results that arise from the study provided such a use is only for scientific purpose. I am aware that participating in this study would imply that I will be cooperating for all clinical and biochemical tests by the principal investigator.

I …… the undersigned , give consent to be a participant of this study.

Signature/Thumb impression of the participant

(Name/Address)

Signature /Thumb impression of the witness

(Name/Address)

Name & Signature of the investigator

KEY TO MASTER CHART

- PUO Pyrexia of unknown origin
- CSOM Chronic suppurative otitis media
- b/l Bilateral
- Hb Hemoglobin
- TC Total count
- DM Diabetes mellitus
- HTN Hypertension
- BA Bronchial Asthma
- ESR Erythrocyte sedimentation rate
- UTI Urinary tract infection
- TB Tuberculosis
- PS Peripheral smear
- BM Bone marrow
- HIV Human immunodeficiency virus
- RTA Road traffic accident
- CAD Coronary artery disease
- CRP C-Reactive Protein
- LDH Lactate Dehydrogenase

Master Chart

Name	Age	Sex	complaints	Hb%	TC	Lymphocyt	Absolute Ly	Neutrophil%	Platelets	Biochemica	Other tests	Clinical dia	Lymphader	Mp/MF	PS	BM	Alcoholism	Smoking	DM/HTN/A	Review PS
Malliga	41	F	generalized	10.8	8800	40	3520	51				Psoriasis			Reactive lymphocytes					Normal
Saroja	64	F	fever, coug	9	7290	44	3207.6	52	2.46	CRP positive		PUO			mature lymphocytes					Normal
Shaffequa Jammal	22	M	fever	10.4	7500	41	3075	54				Fever			Activated lymphocytes					Normal
Sakthivel	39	M	right inguin	14.4	7900	40	3160	57	2.25			Inguinal hernia			Reactive lymphocytes		+			Normal
Farvin begam	28	F	abdominal	12.8	14800	42	6216	54	3.84			fibroid uterus,endometriosis			Reactive lymphocytes					Normal
Satyamoorthy	55	M	bleedingP/I	14.5	6600	52	3432	40	2.11		Serology n	infected rectal polyp			Mature lymphocytes		+	+		Normal
Dhanraj	46	M	Cough with	11	8000	52	4160	39	1.82			bilateral inguinal hernia			Reactive lymphocytes		+	+		Normal
Abdul Salam	55	M	Cough with	14.4	10000	44	4400	53	2.14			bronchiecta	right inguinal positive 1		Normal lymphocytes		+	+		Normal
Sasikala	45	F	Mass P/V	10.9	7100	47	3337	48	2.45			prolapse uterus			Normal lymphocytes					Normal
Kalaiarasi	29	F	swelling rig	11.3	7300	56	4088	42	3.66		urine pus c	epidemal inclusion cyst with UTI			Atypical Lymphocytes			+		Normal
Gokul	16	M	Proptosis	6.9	9180	39	3580.2	47	4.9			Leukemia/l	cervical lymph node +		Neutrophilic	more than 80 % of immature cells, b				
Abdul Hameed	45	M	cough	14	12780	40	5112	49	3.23		mantoux po	Pulmonary TB			neutrophilic leukocytosis , reactive ly		+	+		Normal
Sridevi	30	F	fever,cough	8	8500	60	5100	40	1.48			Bronchial Asthma			mature lymphocytes					Normal
Manjula	27	F	burning mic	12.3	8090	52	4206.8	36	2.35			Monilial vaginosis			Lymphocytic predominance with reactive lymphocytes				DM/ HTN	Normal
Thiyat nayak	63	F	Tiredness/V	11.8	8850	50.7	4486.95	44	2.84			Uncontrolled Type II DM			Lymphocytic predominance with reactive lymphocytes					Normal
Fareesha	42	F	Scalp absc	13.5	16070	29.1	4676.37	62.6	2.9		ESR - 45/9	Scalp abscess			Leukocytosis with neutrophilic toxic changes, mature lymph				DM/ HTN	Normal
Jayalakshmi	15	F	Throat pain	11.7	11290	41	4628.9	53	3.1			Throat infection			Reactive lymphocytes					Normal
Kamalam	48	F	Cough with	11.9	10000	43.9	4390	51.9	3.14			Pulmonary TB			Reactive lymphocytes					Normal
Muthulakshmi	53	F	ear pain, d	11.9	12130	40.4	4900.52	54.5	2.32			CSOM			Reactive lymphocytes				DM/ HTN	Normal
Kamalam	58	F	fever	9.5	7600	56	4256	32		CRP negative		PUO			Reactive lymphocytes				DM/ HTN/ I	Normal
Indhirani	56	F	psycosis	10.6	8870	46.3	4106.81	47.6	2.61			Psycosis			Reactive lymphocytes				DM/HTN	Normal
anandhi	61	F	cough with fever,vomit		5400	80	4320	19				Apalstic anemia			Mature lymphocytes					Normal
Revathy	37	F	fever for ev	11.6	9630	58	5585.4	43	3.74			PUO			Occasional reactive lymphocytes					Normal
Revanth	37	M	Fever, b/l a	12.1	10280	47	4831.6	47	1.21			Fever for evaluation			Reactive lymphocytes with lymphocytic predomi		+			Normal
Maria Anitha	13	F	Abdominal	8.3	7170	53	3800.1	35		CRP positive		fever for evaluation			Mature lymphocytes					Normal
Periasamy	70	M	leg pain	10.5	8210	56.2	4614.02	33.7	2.28			cellulitis, pusc/s- streptococcus			Lymphocytic predominance				DM	Normal
Parvathy	48	F	fever, coug	13.4	9470	42.7	4043.69	48.3	4.03			bronchial asthma on inhaler			Lymphocytic predominane with smudge cells					Normal
Meenammal	60	F	fever for ev	12	8500	48.6	4131	45.3	3.13			viral fever			Reactive lymphocytes				DM	Normal
Rajeshwari	21	F	Tonsillitis	12.3	10230	43.4	4439.82	51.8	2.8			Chronic ton	Cervical node +		mature lymphocytes					Normal
Thomas Mary	46	F	psycosis	7.5	12420	44.7	5551.74	52.2	1.3			Psychiatric illness			Reactive lymphocytes				DM	Normal
Santhosh	13	M	Throat pain	10.7	10050	33.4	3356.7	52.7	3.58			Pharyngitis	Cervical nodes+		mature lymphocytes					Normal
Robert Kennedy	48	M	RTA, Fract	14.3	9460	35.9	3396.14	50.6	2.5			RTA			Reactive lymphocytes		+	+		Normal
Jaya Mary	60	F	Fever for e	11.4	10150	32.4	3288.6	57.6	2.82			tuberculosi	axillary nodes +		Mature lymphocytes				DM	Normal
Chinnaiyar	67	M	Abdominal	12.8	8090	42.5	3438.25	47.6	3.21			Peptic ulcer			Mature lymphocytes		+	+	HTN	Normal
Balachandran	20	M	Abdominal	15	9320	36.8	3429.76	52	3.44			Appendicitis			Mature lymphocytes			+		Normal
Dhilagavathy	46	F	Fever for e	12.5	7000	52.8	3696	38.8	2.31			Viral fever			mature lymphocytes					Normal
Gomathy	33	F	skin lesions	10.1	6980	47.1	3287.58	45.7	2.88			Tuberculos	axillary nodes +		reactive lymphocytes					Normal
Aringer Mani	67	M	chest pain	16	12960	45	5832	46	3.11			Trauma			Reactive lymphocytes		+	+	DM/HTN	Normal
Chinnammal	50	F	Fever, Wea	10.3	7900	53.8	4250.2	39.5	1.5			Viral fever			mature lymphocytes			tobacco ch	DM	Normal
Preethan	33	M	reurrent se	10.5	14370	30	4311	65	3.4			Recurrent seizure disorder/epileps			Reactive lymphocytes					Reactive lymphocytes
Shanthy	22	F	Headache	12.7	8500	51.1	4343.5	43.7	2.46			Migraine			Reactive lymphocytes					Normal
Regina Begam	44	F	psychosis	12.1	9600	52	4992	36	2.37			Psychosis			mature lymphocytes					Normal
Vijayalakshmi	64	F	Fever for e	11.5	9310	42.1	3919.51	51.7	2.67			viral fever			Reactive lymphocytes					Normal
Nithya	23	F	cough with	10.5	17420	36	6271.2	60	3.86		Mantoux +	tuberculosi	cervical lymph nodes +		Reactive lymphocytes					Normal
Jannathu nisha	60	F	Fever for e	11.8	10410	38.7	4028.67	55.7	2.35		Mantoux +	Tuberculosis			Reactive lymphocytes				DM	normal
Thagira Banu	28	F	Discharge	5.9	10290	42.1	4332.09	50.5	4.76			HIV			Atypical lymphocytes					Reactive lymphocytes
Rajendran	31	M	diarrhoea,	10.9	7350	50	3675	41	3.71			possibility of liver abscess			Normal lymphocytes					Normal
Kalpana Devi	23	F	Generalise	6	8460	52	4399.2	46	3.13			Adverse drug reaction			Reactive lymphocytes				DM	Normal
Muthulakshmi	55	F	Prolapse u	9.4	9540	42.4	4044.96	47.6	1.49			Prolapse uterus with chronic cervi			mature lymphocytes			+, tobacco	DM	Normal
Jayanthy	45	F	Trauma	12.4	10500	41.9	4399.5	53.41	1.5			RTA			Reactive lymphocytes					Normal
Kulanji	50	F	generalized	14.5	10480	39.1	4097.68	50.1	3.14			Adverse drug reaction			Reactive lymphocytes					Normal
Banu	42	F	dyspnea, c	11.9	9510	45.4	4317.54	47.7	4.54			bronchial asthma on inhaler			Reactive lymphocytes				BA	Normal
Vimal Nath	45	M	burns	11	10650	45.8	4877.7	45.7	2.25			second degree burns			Reactive lymphocytes					normal
Anjalai	55	F	chest disco	12.7	10260	43.1	4422.06	57.9	2.34	LDH not elevated		Myocardial infarction			Atypical lymphocytes				HTN	normal
Madi Muthu	60	M	generalised	13.9	16860	50	8430	40.5	2.11			Utricaria			Reactive lymphocytes			+	DM	normal
Sameen	36	F	Trauma	11.2	8690	52.1	4527.49	37.1	2.3			RTA, skin lacerations			Reactive lymphocytes					normal
Nagarajan	52	M	Uncontrlled	12.8	9360	45.7	4277.52	39.1	2.98			Uncontrolled DM			Reactive lymphocytes					normal
Selvam	52	M	Tuberculos	13.4	11720	36	4219.2	53	2.51		Mantoux +	Tuberculos	Cervica lymph node +		Reactive lymphocytes		+	+	DM	normal
Jasina begam	42	F	Utricaria	12.7	10030	40.3	4042.09	48.2	3.11			hypersensitivity reactions			mature lymphocytes					Normal
Nagore Gani	52	M	Fever for e	14.7	11260	37.1	4177.46	51	3.21			gastroenteritis			Reactive lymphocytes			+		Normal

Natchiyar	48	F	HIV	11.1	5600	65.6	3673.6	32.3	2.01			HIV +			Atypical lymphocytes				Normal
Karthiga	27	F	Fever for e	13.6	10680	40.4	4314.72	51.5	1.97	CRP negative		Viral fever			mature lymphocytes				Normal
Rasiya Banu	33	F	RTA, head	12.7	11990	43.3	5191.67	49.1	3.05			RTA			Reactive lymphocytes				Normal
Hyadar Ali	52	M	chest pain,	10.4	10130	40	4052	47.9	3.32			Acute cardiac failure			Reactive lymphocytes		+	DM	Normal
Sulochana	38	F	Trauma, tib	12	13830	30	4149	62	2.71			Tibial fracture, Trauma			Reactive lymphocytes				Normal
Rengaraj	35	M	pain, multip	15.6	10360	40	4144	55	2.91			Rheumatoid arthritis			Reactive lymphocytes		+		Normal
lakshmi	48	M	RTA	13.7	11770	37.4	4401.98	58.4	2.69			RTA			Reactive lymphocytes		+		Normal
Arulmary	42	F	Chest pain	11.2	14490	36.5	5288.85	58	3.2	LDH elevated		Myocardial infarction			Reactive lymphocytes			HTN	Normal
Pavithra	20	F	Generalise	12.4	11340	38.1	4320.54	54.3	3.75			utricaria			reactive lymphocytes				Normal
Siva nandhan	57	M	Master hea	11.5	10500	50.8	5334	44.3	2.77			Diabetes mellitus			Reactive lymphocytes		+	DM	Normal
Jaya	17	F	abdominal	11.7	11000	39.2	4312	53.2	3.11			Acute appendicitis			reactive lymphocytes				Normal
Magamayee	65	F	Headache	11.6	12040	44.6	5369.84	51.2	2.29			stress induced headache			reactive lymphocytes			DM/HTN	Normal
Satya	40	M	Trauma	12.3	13650	32.2	4395.3	58.4	5.38			RTA			Mature lymphocytes		+		Normal
Meharrnisha	47	F	Discharge	12.3	11430	40.4	4617.72	54	2.67			Viral fever, candidiasis			reactive lymphocytes			DM	Normal
Poomalai	57	F	Master hea	12.4	11500	41.6	4784	41.5	2.85			DM			Mature lymphocytes			DM	Normal
Backiyaraj	52	M	Leg pain	12.1	11270	42.7	4812.29	51.9	1.9			Trauma			Mature lymphocytes		+	DM/HTN	Normal
Vencila mary	22	F	Acute abdo	8.7	12210	41.2	5030.52	45.7	3.65			pancreatitis			reactive lymphocytes				Normal
Kalavathy	45	F	Cough with	13.1	8080	42.3	3417.84	44.3	1.92		Mantoux +	Tuberculosis			Mature lymphocytes			DM	Normal
Santham	21	M	h/o tiredne	9.8	6000	50.8	3048	40	1.48			Tuberculosis			reactive lymphocytes		+		Normal
Sivagami	80	F	uncontrolle	9.1	10310	53	5464.3	39	2.81			Uncontrolled DM			Mature lymphocytes			DM/HTN	Normal
Uma devi	35	F	master hea	8.6	6600	46	3036	47	3.06			BA on inhalers			Mature lymphocytes			DM/BA	Normal
Margred	28	F	master hea	10.7	7880	41.8	3293.84	47.3	1.95			Fever			reactive lymphocytes				Normal
Lakshmi	40	F	Fever for e	12.9	8330	44.3	3690.19	50	3.09			viral fever			Mature lymphocytes				Normal
Ramu	50	M	cough with	10	6770	48	3249.6	42	2.43		Mantoux +	Tuberculoisis			Mature lymphocytes		+		Normal
Birla	35	M	master hea	16	7400	43	3182	45	2.13						Mature lymphocytes		+		Normal
Sirumbayee	65	F	Trauma	10.8	8820	43.2	3810.24	42.3	2.52			fracture neck of femur			Mature lymphocytes			DM	Normal
Karpagam	19	F	Fever with	8.1	7789	42.2	3286.958	50.4	2.53			Malaria		+	Reactive lymphocytes				Normal
Mariya Louis	47	M	fever with l	3	6500	48	3120	40	1.65			dimorphic anemia with lymphocytic			Mature lymphocytes		+		Normal
Selvi	45	F	abdomial p	11.8	10350	42.9	4440.15	50	3.14			Endometritis			Reactive lymphocytes				Normal
Banumathy	41	F	Fever, cou	12.7	8100	47	3807	42	2.45			Tuberculosis			Reactive lymphocytes				Normal
Shahul Hameed	27	M	cough	15.1	8900	48.5	4316.5	45.2	2.75			pharyngitis			Reactive lymphocytes		+		Normal
Chellammal	40	F	fever	11.9	6500	47.1	3061.5	43	2.25			tonsillitis			Mature lymphocytes				Normal
Kamalam	48	F	burns, oil s	11.9	10090	42	4237.8	51	3.14			burns			Reactive lymphocytes				Normal
Banumathy	52	F	fever	12.1	11270	42.7	4812.29	51.9	2			PUO			Mature lymphocytes			DM	Normal
Fathima	31	F	bleedind P	9.5	11350	30.2	3427.7	53.5	1.77			Endometritis			Mature lymphocytes				Normal
Ramachandran	46	M	Cough with	17.5	13270	26.7	3543.09	66.2	1.8		Mantoux +	Tuberculosis			Reactive lymphocytes		+		Normal
Selvambal	59	F	Fever	11.3	13790	30	4137	65.1	3.39			Viral fever			Mature lymphocytes			DM	Normal
Joseph	60	M	Cough	14.5	8690	40.2	3493.38	48.8	2.29						Mature lymhocytes		+		Normal
Subulakshmi	50	F	Cough with	12.5	8660	40	3464	48	2.58			Pharyngitis			Mature lymphocytes			DM	Normal
Sivakumar	45	M	Cough	14.1	8002	41.3	3304.826	52.6	2.89						Mature lymphocytes	+	+		Normal
Leelamary	68	F	Fever	11.8	12280	26.2	3217.36	67.2	2.4			PUO			reactive lymphocytes				Normal
Ananthan	43	M	cough with	14.7	13050	42	5481	50	3.71			Pharyngitis			Reactive lymphocytes		+		Normal
Rajeshwari	32	F	Seizures	12.1	10910	39.4	4298.54	55.1	4.6			Recurrent seizures			Reactive lymphocytes				Normal
Sanjai	46	M	Headache	11.5	7300	46.7	3409.1	45.2	2.48			migraine			Occasional reactive lymphocytes		+		Normal
Siva	36	M	Fever	15.5	10860	50.7	5506.02	45.9	0.95		dengue IgM	dengue			Reactive lymphocytes				Normal
Vaitha Beevi	45	F	breathlessr	14.9	8990	46.2	4153.38	49	3.09	LDH not elevated		CAD			Reactive lymphocytes			DM	Normal
Saroja	48	F	fever	12.3	9290	44.6	4143.34	50.2	2.56	CRP negative		PUO			Reactive lymphocytes			HTN	Normal
Rasammal	50	F	seizures	11	11260	39.5	4447.7	52.2	3.21			Seizure disorder			Reactive lymphocytes			DM	Normal
Balasubranmaniya	15	M	fever	14.1	9100	50.2	4568.2	43.3	2.71			Fever for evaluation			mature lymphocytes				Normal
Chellammal	22	F	Psychiatric	9.5	8860	43	3809.8	47.4	3.98			Psychosis			Reactive lymphocytes				Normal
Selvaraj	26	M	RTA	15.4	12180	43.5	5298.3	45.8	2.44			RTA			Reactive lymphocytes				Normal
Jeevananthan	45	M	cough with	12.7	11280	37.1	4184.88	52.9	2.25		Mantoux +	tuberculosis			Reactive lymphocytes				Normal
Padmavathy	29	F	Cough	13.3	12460	39.4	4909.24	52.1	2.52			Cough for evaluation			Reacive lymphocytes				Normal
Eashwaran	28	M	fever with	16.9	27950	23.2	6484.4	69.7	3.27			Fever for evaluation			Mature lymphocytes				Normal
Jayanthi	35	F	giddiness	11.3	11940	34	4059.6	59	2.79			uncontrolled DM and hypertensio			Reactive lymphocytes			DM /HTN	Normal
Thayammal	48	F	fibroadeno	11.1	6520	50.3	3279.56	43.1	1.99			Fibroadenoma breast			Reactive lymphocytes				Normal
Meena	52	F	Fever with	14.6	19060	23	4383.8	51	3.34			? Tuberculosis			Mature lymphocytes			DM	Normal
Nandhini	18	F	Fever with	12.8	12460	27.1	3376.66	65.4	3.14	CRP negative		Fever for evaluation			Mature lymphocytes				Normal
Saravanan	32	M	Fever	15.1	8400	48.6	4082.4	43.1	2.14		dengue IgM	dengue			Reactive lymphocytes				Normal
Ishagani	35	F	Abdominal	10.3	14170	30.2	4279.34	64	3.28		serum amy	acute pancreatitis			Mature lymphocytes				Normal